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EFFECTS OF ULTRAVIOLET RADIATION UPON REPRESENTATIVE SPECIES OF FUSARIUM[1]

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(WITH PLATE I AND SIX FIGURES)

Introduction

One of the most common fungi with which the market pathologist must deal is the ubiquitous *Fusarium*. There are very few vegetables on which *Fusarium* does not occur, either as a primary or secondary incitant of decay, and so the problem of identification of *Fusarium* species is an ever present one. During the course of recent work with species of *Fusarium* isolated from decayed onion bulbs, one of the chief difficulties has been the production in artificial culture of sufficient normal macrospores for taxonomic studies. Various methods of inducing sporulation have been tried, but in many cases these were unsuccessful or only inconsistently fruitful. Often microspores were produced in abundance but the macrospores, which are usually necessary for identification, were lacking. The consistent success of RAMSEY and BAILEY (**14**) in producing macrospores of the onion parasite *Fusarium cepae* Hanz. emend. LINK and BAILEY (**8**) upon irradiation with ultraviolet suggested the use of this method for unidentified strains of *Fusarium* from onion. In not all instances was the irradiation as beneficial as in the case of *F. cepae*, but in a large percentage of strains it was helpful and in some it induced marked increase in spore production.

[1] Contribution from the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Department of Botany, University of Chicago, cooperating.

Because of the value of ultraviolet radiation in the work on the taxonomy of onion Fusaria, irradiation of authentic species of *Fusarium* from the various sections of the genus was tried, in the hope that this might prove helpful in the ever difficult problem of taxonomic diagnosis of the many species within the form genus. Investigations on the effect of ultraviolet irradiation upon members of the genus *Fusarium* have been made by others, but most of the work has dealt with the lethal action of the rays. SIBILIA (**18**) describes the lethal effect of ultraviolet on two species of *Fusarium*, *F. echinosporum* and *F. fuliginosporum*, first described by him (**17**) but not mentioned by WOLLENWEBER in the Fusaria Autographice Delineata (**29, 30**). SIBILIA found that exposure of the conidia of these two strains in drops of distilled water or of 1 per cent glucose solution at a distance of 12 cm. from the unscreened quartz mercury vapor lamp for two minutes or over invariably resulted in death. The age of the conidia seemed to play no part in their resistance to ultraviolet. Chlamydospores were more resistant than conidia. In most of the experiments, exposures as short as one second, even at 90 cm. from the arc, resulted in death; however a distance of 2 meters permitted germination of the spores, the air screening out the more detrimental rays. The contents of spores which were killed by radiations were greatly plasmolyzed, and showed numerous small globular masses or, rarely, a single ellipsoidal mass. Conidia germinating under irradiation had abnormal germ tubes, often contorted and soon arrested in growth.

FULTON and COBLENTZ (**5**), working with *Fusarium* sp. from orange fruit, found that the spores, in water suspensions, were killed by 1-minute exposures 6 inches from an unscreened 110-volt quartz mercury burner having a mercury cathode and a tungsten anode, operated on 320 watts (80 volts, 4 amperes). Mycelium from germinated spores was more easily killed than were ungerminated spores of the various fungi tested. The fungicidal effectiveness of treatment with ultraviolet depended on the intensity of the radiation and on the wave lengths of the component rays; the shorter the wave length the more lethal the effect with a given intensity. The killing effect was cumulative, several short intermittent exposures (rest periods were the same length as exposure periods) having almost the

effect of one continuous exposure of the same total time. The killing effect increased with the lengthening of the time of action, and also with the increase in intensity of irradiation.

DILLON WESTON and HALNAN (**4**) also worked on *Fusarium* sp. and found that the mycelium was not killed but that growth was inhibited. Their tests were not run with an unscreened lamp, however, but with vitaglass and Sanalux petri dish covers transmitting only 50 to 70 per cent of the ultraviolet light from a quartz mercury-vapor lamp (Hanovia artificial alpine Sun, 2.5 amp. 210 v. direct current type). Potato agar plates were inoculated from a pure culture of the *Fusarium*, a needle being used to carry the spores and mycelium into the medium. Irradiation was commenced the same day and was continued for a fortnight with daily 9-minute exposures. They found that the mycelium of *Mucor* and *Fusarium* grew farther down into the medium in the irradiated plates; "consequently, aerial development is retarded and the sporangiophores and conidiophores appear stunted." After the first week of irradiation the plates were reversed and the unirradiated controls were subjected to a week's treatment while the previously treated plates were held as controls. "The effect of this treatment was to inhibit the growth of the original controls and to encourage growth in the culture that had originally been retarded by the action of the light." They suggest that in many cases the mycelium may not be killed but rendered dormant for some considerable time.

STEVENS (**21**), in a group of fungi with which he says he obtained no interesting results from irradiations of 10, 30, and 50 seconds on corn meal agar "except stunting of colony and perhaps darkening and distortion or suppression of spore structures," lists: *Fusarium batatatis*, *F. conglutinans*, *F. niveum*, *F. oxysporum* (faint reddening on irradiation), *F. vasinfectum*, and *Fusarium* spp. (many). He also irradiated *Gibberella saubinetii*, which has as a conidial stage *F. graminearum*, and obtained with irradiations of 30 and 50 seconds distinct reddening of the mycelial tip, although he does not report the production of perithecia as in the case of *Colletotrichum lagenarium* (**25**).

JOHNSON (**7**) found that the aerial mycelium of *F. batatatis* Wr. collapsed, and that there was an increase in pigment within 24

hours after a 10-minute irradiation with Schumann rays. The collapsed mycelium "revealed a very granular protoplasm and the granules were relatively large." The mycelium was not entirely killed by a 25-minute exposure. Both macrospores and microspores occurred on controls but were more abundant after irradiation. Eight-day old cultures were less susceptible than those 5 days old. He found, also, that the ultraviolet transmitted by a Hanovia quartz mercury-arc lamp through cellophane (5790–2201 A.u.) produced no lethal effects on spores newly planted on agar, even if the dosage was as much as 50 minutes at 8 inches from the lamp. Three to 4-day old cultures given five daily exposures of 5–10 minutes through cellophane at 8–16 inches from the arc showed a "pronounced increase in pigment and a very noticeable suppression of growth." Zonation of the colony also occurred. The same types of reactions occurred to a less degree with filters transmitting to 2302 and 2536 A.u. With filters transmitting no lower than 2907 A.u. no variation was present after exposures as long as 3 hours at 8 inches.

RABINOVITZ-SERENI (**13**) worked with *Fusarium martii* and *F. poae*, and found that the spores of neither species were very resistant to the action of the ultraviolet emitted by a Gallois di Lione quartz mercury-vapor lamp at 20-cm. distance. Only a small percentage of those irradiated for 12 minutes or more germinated, and the germ tubes of these were abnormal.

Although much attention has been centered on the fungicidal action of short waves, some work has been published on the stimulating action of ultraviolet rays on fungi. NADSON and PHILIPPOV (**9**) reported a stimulating effect of ultraviolet rays on the growth of *Saccharomyces cerevisiae* and *Mucor genevensis* Lendn. In a subsequent paper (**10**) they reported work on four species of yeast and on *M. guilliermondii* Nad. & Phil. The fungi were exposed in petri dishes covered with a screen of thick glass or metal, in the center of which was a small round opening; the lamp used was a Bach quartz mercury-vapor model (100–120 volts, 3.8–4 amp.) transmitting from 2200 A.u. to 5700 A.u. Exposures of 10–20 minutes at 30 cm. from the arc were made 24 hours after inoculation of the plates with water suspensions of the organisms, and several days later it was found

that the part of the cultures submitted to direct radiation was dead, a sterile circle corresponding to the opening in the center of the screen. The rest of the colony, protected by the screen, continued to develop normally except in a narrow zone surrounding the sterile area, in which zone the yeast, influenced by the oblique rays, showed an intense multiplication and growth. Abnormal budding also occurred. In this "zone of stimulation" *M. guilliermondii* produced sporangia in great numbers although zygospores were lacking. In *M. genevensis*, on the other hand, it was the sexual reproduction which was induced in this area, the increased number of zygospores forming a black border to the sterile zone.

PORTER and BOCKSTAHLER (**11**) exposed *Colletotrichum lindemuthianum* and *Cephalothecium roseum* to wave lengths of 3630–3650 A.u. transmitted through a filter by a Cooper-Hewitt quartz mercury-arc lamp 60 cm. distant. They report "inhibition of growth and collapse and probably death of the aerial hyphae," and say: "In addition to these effects spore formation is inhibited sharply in *Cephalothecium* but on the other hand slightly accelerated in the case of *Colletotrichum*."

In the case of *Colletotrichum phomoides*, HUTCHINSON and ASHTON (**6**) were able to accelerate sporulation and retard growth by exposure of 3-day old cultures 12 cm. from the full mercury arc (Cooper-Hewitt) for 15 seconds to 2 minutes. Acervuli formed 24 hours after irradiation, with maximum development taking place in cultures irradiated for 30 seconds to 1 minute. Similar effects could be obtained by irradiating spores previous to planting on agar plates.

STEVENS, in works quoted and in subsequent papers (**22, 23, 24**), reports that he was able to induce pycnidia formation in such forms as *Camerosporium* sp. and in some strains of *Coniothyrium*, and to produce perithecia and acervuli in some strains of *Glomerella cingulata*. Doses approximately 300 times longer were required to kill conidia than were needed to induce perithecia of the last-named fungus. There was no such regularity in the induction of acervuli of *Glomerella cingulata* as in the case of perithecia. Both acervuli and perithecia failed to appear on irradiation of some strains.

DILLON WESTON (**3**) found that the retardation of growth result-

ing from exposure to ultraviolet wave lengths shorter than 400 mμ "may result subsequently in an acceleration of the development of the organism." Cultures of *Fusarium culmorum* were given one irradiation for different periods with light from a Hanovia sun-lamp. Although the primary reaction was one of retardation, subsequently the irradiated cultures sporulated abundantly whereas the non-irradiated cultures sporulated sparsely. In correlating these two facts he states, "it is assumed here that the initial retardation forces the organism to an earlier maturity."

The present paper attempts to make a comparative study of the effects of irradiation upon sporulation and upon certain physiological responses, such as color production and growth rate, in representative species from the described sections of the form genus *Fusarium*. All the sections are represented except section II, Macroconia, section IV, Submicrocera, and section V, Pseudomicrocera, of which no authentic cultures were obtained. All subsections are represented except Aquaeductuum under section Eupionnotes, which contains those species formerly placed in section Camptospora Wr. (**29**). The systematic arrangement followed is that given by WOLLENWEBER in his revised Delineata (**30**) and in the *Fusarium* monograph (**32**).

An attempt has been made to select, so far as possible, species which were typical for each section. Tests were made also with some of those having known perfect stages, in order to see whether irradiation would serve to stimulate production of the perithecial stage. More species were chosen from section Elegans (**28**, p. 28; **31**, p. 741) than from any other section, because this group contains more of the important plant parasites and because there is often greater difficulty in producing macrospores, and in distinguishing some of these forms, than is the case with species from the other sections.

Most of the cultures used were obtained from Dr. H. W. WOLLENWEBER or from Dr. O. A. REINKING at the time of the *Fusarium* conference in Madison, or are cultures brought to the conference by the writer and used in the studies (**34**) there. Credit is given in a footnote connected with table I for authentic cultures obtained from other sources.

Methods

Since, in the experiments with *F. cepae* and *Macrosporium* (**14**), irradiation through vitaglass covers transmitting to 2650 A.u. had proved most satisfactory, this glass was employed in most of the present experiments. Five-inch vitaglass squares were fitted as covers by molding paraffin rings about the edges of petri-dish bases. The lower half of the petri dish was held tightly against the vitaglass square until the paraffin had set, and then was slipped out. A little vaseline was mixed with the paraffin to make it less brittle when cold. These covers were sterilized with alcohol, and when dry were used to replace the ordinary petri-dish lid over the fungus culture previous to its exposure to ultraviolet.

In some of the experiments, a Stewart health-lamp equipped with therapeutic "A" carbons was used and operated on alternating current at 12 amperes through 110-volts resistance. This lamp is equipped with a Corex D filter which transmits traces between 2700 and 2800 A.u., feebly between 2800 and 2850 A.u., and absorbs none of the wave lengths above 2880 A.u. When the filter is not used the lamp gives some feeble transmission as low as 2300 A.u. Increased sporulation may be obtained with the Corex D filter, but in order to get the full effect of the lower wave lengths transmitted by the vitaglass it was necessary to remove the filter from the lamp. With the filter removed the heat produced tended to melt the paraffin rings if the plates were exposed for a long time at a distance of 40 cm. from the arc. For this reason most of the comparative studies were made with a Cooper-Hewitt quartz mercury-arc lamp, which does not produce so much heat. The mercury lamp was operated on alternating current at 4 amperes through resistance from a 110-volt line.

Plantings were made on potato dextrose agar, which had been found to be a very suitable medium for all species tested. For the purpose of these experiments it made little difference whether the inoculum used was a small piece of mycelium or a spore dilution, providing the same type of inoculum was used for control and test plates. The fungi were allowed to grow for four days before irradiation was begun. Some trials were made in which irradiation was begun on the second day, but the results were never so satisfactory.

If the cultures were much older than four days at the beginning of the tests, the mycelium often filled the plates before the end of the tests, thus hindering the measurement of growth rate.

Except in the experiments with various filters and in some of the direct exposures attempting to produce the sexual stage, the exposures were made through vitaglass covers 40 cm. from the unscreened mercury arc. Unless otherwise stated, all cultures were exposed for 15 minutes for each of three successive days. In all tests several plates were used for each exposure, and the average reading of many mounts made for total number of microspores and macrospores present, the results being recorded in terms of macrospore percentage of the total. Controls were held for an equal length of time in the diffused light of the laboratory, and the exposed plates were held in the same place as the controls when not actually under the lamp. All experiments were duplicated and questionable ones checked several times.

Results of irradiation through vitaglass

That not all species of *Fusarium* responded to irradiation with increased macrospore production, as did *Fusarium cepae*, is apparent from table I, which gives a summary of some of the experiments in which the cultures were irradiated through vitaglass for 15 minutes on each of three successive days. In most of the species exposed to ultraviolet there was an increase in total sporulation, if not in the percentage of macrospores produced. In some species, as *F. orthoceras* App. and Wr. var. *albidoviolaceum* (Dasz.) Wr. (Section XIV), the irradiated plates, although showing very few macrospores (fig. 1 *E*), actually had such an increase in microspore production as to produce an exposed pionnotes or at least marked zonation of the colony (fig. 1 *A*), while the control plates showed only aerial microspores and non-zonate mycelium. On the other hand, in forms like *F. stilboides* var. *minus* Wr. (Section XII), where microspores are usually lacking or scarce and where the percentage of macrospores present is therefore high in the control plates, the amount of macrospores in the irradiated plates was often increased to form sporodochia (fig. 1 *B*) or a definite exposed pionnotes, as contrasted with few spores present in the controls. Usually increase in sporula-

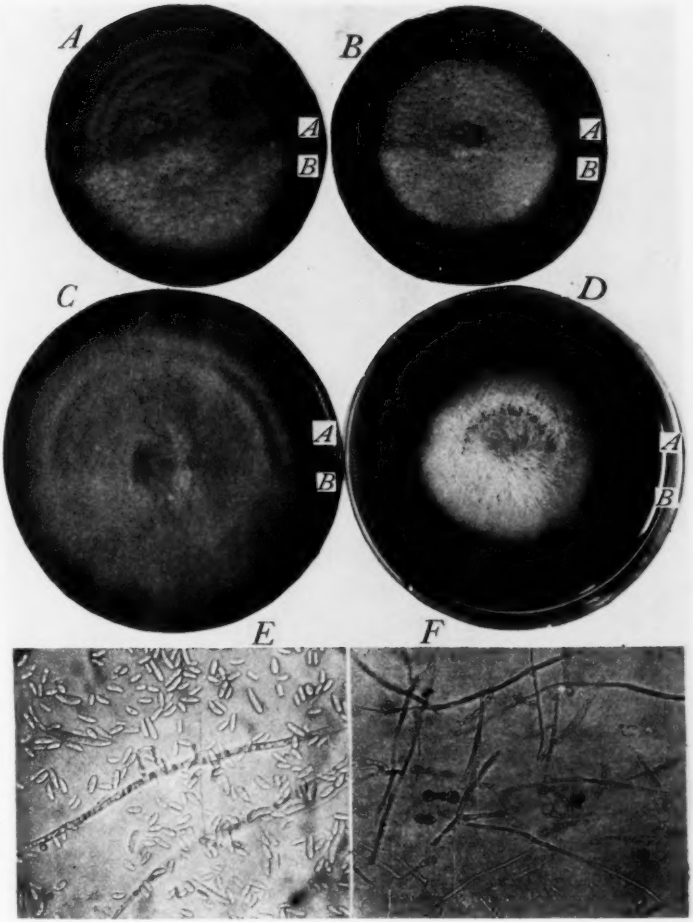


FIG. 1.—A–D, petri-dish cultures of *Fusarium* spp. showing effects of three daily 15-minute exposures to ultraviolet irradiation through vitaglass (2650–6690 A.u.), 40 cm. from quartz mercury arc: A, *F. orthoceras* var. *albidoviolaceum:* (a) irradiated portion showing zonate growth and deeper color due to sporulation; (b) control half (covered with photographer's black paper during irradiation); B, *F. stilboides* var. *minus:* (a) irradiated portion showing sporodochia; (b) control half; C, *F. nivale:* (a) irradiated portion showing more powdery appearance and zonation; (b) control half; D, *F. dimerum:* (a) irradiated portion showing exposed pionnotes; (b) control portion; E, *F. orthoceras* var. *albidoviolaceum* showing scarcity of macrospores after three daily 15-minute exposures through vitaglass; F, *F. argillaceum* showing injured spores and mycelium and chlamydospores produced on old portion of colony which had received three daily 15-minute exposures through vitaglass.

TABLE I

SUMMARY OF EXPERIMENTS SHOWING EFFECTS OF THREE SUCCESSIVE 15-MINUTE EXPOSURES TO ULTRAVIOLET TRANSMITTED THROUGH VITAGLASS (LOWER LIMIT OF TRANSMISSION, 2650 A.U.)

SECTION	SPECIES OF FUSARIUM	PERCENTAGE MACROSPORES		OTHER DIFFERENCES
		IRRADIATED	CONTROL	
1. Eupionnotes	F. dimerum Penz. (*R*)*	0.0	0.0	More microspores and deeper color on exposed plates
	F. merismoides Cda. var. majus Wr. (Syn. F. betae (Desm.) Sacc. (*W*)	10.9	7.7	Total sporulation (macro- and microspores) greater on tests
2. Macroconia	No representative	.....	.....	.....
3. Spicarioides	F. decemcellulare Brick. (*R*)	1.0	−1.0	More microspores on exposed
4. Submicrocera	No representative	.....	.....	.....
5. Pseudomicrocera	No representative	.....	.....	.....
6. Arachnites	F. nivale (Fr.) Ces. (*D–L*)	68.5	53.5	Exposed culture more powdery
7. Sporotrichiella	F. sporotrichioides Sherb. (*B*)	60.7	32.8	Thicker pionnotes on exposed plates
8. Roseum	F. anguioides Sherb. (*R*)	6.0	−1.0	.....
9. Arthrosporiella	F. semitectum B. & Rav. (*W*)	23.7	19.4	
	F. semitectum var. majus Wr. (*R*) (Syn. F. incarnatum (Rob) Sacc.)	94.3	64.1	Thicker pionnotes on exposed plates

* Letters in italics refer to source of the culture as follows:

B, Writer's culture
C, G. H. COONS
D–L, A. S. DAHL through G. K. K. LINK
G, W. W. GILBERT
H, L. L. HARTER
J, HELEN JOHANN
R, O. A. REINKING
S, M. SHAPOVALOV
T–N, J. J. TAUBENHAUS through A. G. NEWHALL
W, H. W. WOLLENWEBER
W–J, WOLLENWEBER through HELEN JOHANN
Wis, Wisconsin laboratory
W–L, Wisconsin laboratory through G. K. K. LINK

TABLE I—*Continued*

10. Gibbosum	F. equiseti (Cda) Sacc. f. 1 Wr. (*R*) (Syn. F. ossicolum (B & C) Sacc.)	37.7	27.0	Exposed pionnotes on irradiated plates
	F. scirpi L. & F. var. acuminatum (E. & E.) Wr. (*R*)	89.0	50.0	Very few spores in control; more plentiful on exposed
	F. scirpi L. & F. var. longipes (Wr. & Rg.) Wr. (*R*)	51.7	38.1	
	?F. scirpi L. & F. var. compactum Wr. (*B*)	75.0	0.0	See text
	?F. scirpi L. & F. var. compactum Wr. 2nd test after spores were once obtained by irradiation	71.4	69.0	See text
11. Discolor ss. Neesiola	F. heterosporum Nees. var. paspalicola (P. Henn.) Wr. (*W*)	1.8	0 to −0.5	Total sporulation greater after irradiation
ss. Trichothecioides	F. trichothecioides Wr. (*B*)	5.1	−1.0	A good many 2-septate spores on irradiated plates
ss. Saubinetii	F. macroceras Wr. & Rg. (*R*)	−0.5	−0.5	Total sporulation greater on exposed plates
	F. culmorum (W. G. Sm.) Sacc. (*W–J*)	83.2	0.0	No microspores on controls
	F. sambucinum Fuckel (*S*)	93.8	91.0	Irradiated plates and controls look alike
	F. sambucinum Fuckel (*B*)	95.0	0.0	No microspores on controls; few spores on treated plates
12. Lateritium	F. stilboides var. minus Wr. (*R*) (Syn. F. fructigenum Fr. var. maius Wr. f. 1 Wr. & Rg.)	93.2	81.8	Spores rare in control; plentiful in definite pionnotes on exposed plates

TABLE I—*Continued*

Section	Species of Fusarium	Percentage macrospores		Other differences
		Irradiated	Control	
13. Liseola	F. neoceras Wr. & Rg. (*R*)	35.2	3.7	Greater sporulation on exposed plates
	F. moniliforme Sheld. (*R*)	−1.0	0	" " " " "
	F. " " var. subglutinans Wr. & Rg. (*R*)	6.0	3.2	" " " " "
	F. moniliforme Sheld. var. erumpens Wr. & Rg. (*R*)	−1	−0.5	" " " " "
14. Elegans ss. Orthocera	F. orthoceras App. & Wr. var. longius (Sherb) Wr. (*W*)	4.4	3	
	F. orthoceras var. albidoviolaceum (Dasz) Wr. (*C*)	−1	−0.5	Exposed pionnotes and many microspores on irradiated plates; no pionnotes and few microspores on controls
	F. orthoceras var. albidoviolaceum (Syn. F. asclerotium) (*strain from W*)	1.7	0	Total sporulation greater on irradiated plates
	F. orthoceras var. triseptatum Wr. (*R*)	−1	−1	Thin pionnotes on exposed plates; no pionnotes and few microspores on controls
	F. bostrycoides Wr. & Rg. (*R*)	−0.5	−0.5	Exposed culture more powdery
	F. conglutinans Wr. (*Wis*)	−1.0	0.0	Total sporulation greater on irradiated plates
	F. " " var. callistephi Beach (*C*)	7.4	−0.5	Exposed pionnotes on irradiated plates; not on controls
ss. Constrictum	F. bulbigenum Cke. et Mass. f. 1 Wr. (Syn. F. lycopersici) (*W*)	−1	0	Thicker pionnotes and deeper color than controls
	F. bulbigenum Cke. et Mass. f. 1 Wr. (Syn. F. lycopersici) (*strain 2-W*)	4.3	3.7	As above; strains from 2 other sources acted the same
	F. bulbigenum var. batatas Wr. (Syn. F. batatatis Wr.) (*H*)	4.8	0.8	Relatively few spores in controls; many in treated plates

TABLE I—*Continued*

ss. Oxysporum	F. oxysporum Schlect. (*R*)	−1	−0.5	
	F. " " (*2nd strain S*)	2.3	−0.5	Total sporulation greater on irradiated plates
	F. " " f. 1 Wr. (*W*) (Syn. F. euoxysporum Wr.)	2.2	0	As above
	F. oxysporum f. 2 Wr. (*S*) (Syn. F. hyperoxysporum Wr.)	2	−1	Thinner pionnotes on controls; 2 strains from Harter acted same way
	F. oxysporum f. 3 Wr. (Syn. F. cubense E.F.S.) (*2 strains R*)	Strain A, 8 Strain B, 1	0 0	No pionnotes on control; thin one on irradiated plates
	F. oxysporum f. 5 Wr. (*R*) (Syn. F. oxysporum var. nicotianae Johns)	69	28.3	Pionnotes thinner and covered with mycelium in controls
	F. oxysporum var. aurantiacum (Lk.) Wr. (Syn. F. aurantiacum) (*W*)	7.6	0	Total sporulation greater on exposed plates
	F. cepae Hanz. emend. Link & Bail.	51.0	5.0	Exposed pionnotes on irradiated plates
	F. vasinfectum Atk. (*G*)	1	1	Total sporulation greater
	F. " " f. 1 Wr. (Syn. F. vas. var. inodoratum) (*W*)	96.9	83	More mycelium in controls
	F. vasinfectum var. lutulatum (Sherb.) Wr. (*R*)	55.5	42.4	No aerial mycelium on exposed plates
	F. vasinfectum var. zonatum (Sh) Wr. (Syn. F. zonatum) (*W*)	28	4.9	Pionnotes thicker on exposed plates
	F. vasinfectum var. zonatum f. 1 (Lk. & Bail.) Wr. (*B*)	81.9	69	" " " " "
	F. vasinfectum var. zonatum f. 2 (Lk. & Bail.) Wr. (*B*)	59.0	51.2	" " " " "
	F. redolens Wr. (*W*)	83.9	53.4	" " " " "
15. Martiella	F. coeruleum (Lib) Sacc. (*S*)	96.8	0	No spores of any kind on controls; pionnotes on irradiated plates
	F. solani App. & Wr. var. medium Wr. (Syn. F. malli Taub.) (*T–N*)	59.1	3.4	Exposed pionnotes on irradiated plates
	F. javanicum Koord. (*R*)	39.9	17.0	See text
16. Ventricosum	F. argillaceum (Fr.) Sacc. (*W–J*)	73.2	65.9	Exposed plates show appressed moist mycelium, much stunting and many chlamydospores

tion was noticeable macroscopically in production of sporodochia or pionnotes, or at least in greater intensities of color in mycelium, stroma, and spore mass. In *F. nivale* (Fr.) Ces. (Section VI), although the increase in macrospore percentage was not marked, the treated colonies were much more powdery owing to the increased sporulation (fig. 1 *C*). In such species as *F. dimerum* Penz. (Section I), where macrospores do not occur, an increase in the microspore production resulted in an exposed pionnotes on irradiated plates (fig. 1 *D*), but there was no evident increase in septate over continuous spores. This increase was accompanied by an intensification of the mycelium color. RABINOVITZ-SERENI (**12**) reports such an intensification of colors in cultures of *F. poae* exposed to ordinary blue light, but does not report any associated increase in quantity of spores present.

In some species the macrospore percentage in exposed plates was more than double that in the control plates. In *F. neoceras* Wr. and Rg. (Section XIII) there was an increase from 3.7 to 35.2 per cent; *F. cepae* (Section XIV) increased from 5 to 51 per cent macrospores; and *F. solani* App. and Wr. var. *medium* Wr. (Syn. F. *malli* Taub.) (Section XV), which had given very few normal macrospores in ordinary laboratory culture, showed an average of 59.1 per cent macrospores after irradiating for 15 minutes through vitaglass on three successive days. In most species the response to irradiation was relatively consistent although the percentages varied. For instance, it will be noted (table I) that the average percentage of macrospores present in the *F. sporotrichioides* Sherb. (Section VII) plates after irradiation was 60.7, not quite double that in the control plates, whereas in the experiments summarized in table III the average macrospore production under vitaglass was 51.7 per cent, or five times that of the controls; but in all experiments there was a decided increase in percentage of macrospores. The average percentage for all the experiments with *F. vasinfectum* Atk. var. *zonatum* (Sh.) Wr. (Section XIV), when treated to three successive 15-minute exposures under vitaglass, was 12.1 as against an average of 3 per cent in the controls. Individual tests varied from 8 per cent in the irradiated plates with 0.5 per cent in the controls to 46.7 per cent in the irradiated plates with 5 per cent in the controls. Out of

ten such experiments with this species, each involving several plates, the percentage on the irradiated plates was usually five or six times that from the controls, was never less than three times, and was only once as high as ten times that obtained in the controls.

On the other hand, in an equal number of experiments with *F. conglutinans* Wr. and with *F. bulbigenum* Cke. et Mass. forma I Wr. (syn. *F. lycopersici* [Sacc.] Wr.) and others, the percentage was never increased by as much as 1 per cent under this treatment. The tendency to show definite, unmistakable macrospore production when exposed to the ultraviolet rays transmitted by a quartz mercury arc through vitaglass seemed to be characteristically present or absent for any one of the species tried in these experiments with but few exceptions. The exceptions were *F. scirpi* L. and F. var. *acuminatum* (E. and E.) Wr. (Section X), *F. javanicum* Koord. (Section XV), and *F. argillaceum* (Fr.) Sacc. (Section XVI).

In *F. scirpi* var. *acuminatum* most of the experiments showed striking increase in macrospore content after irradiation, but in two experiments the increase was slight, and in one the controls averaged a higher percentage than the treated plates. In this latter case, however, the number of spores actually present on the control plates was so small as to require examination of a great many mounts before sufficient spores were obtained for counting, whereas in the exposed plates the spores were plentiful although low in macrospore count. Where spores are rare, one would expect a high macrospore count with *F. scirpi* var. *acuminatum*, for even when not in "hochkultur" it produces few microspores. However, the macrospores in control cultures made from subcultures of a strain which had been carried long in artificial culture were often small 3–4 septate spores as contrasted with the well shaped normal 5-septate spores of the irradiated cultures.

F. javanicum is another species which ordinarily produces very few microspores, but the strain which had been received from REINKING in 1924 had been carried in artificial culture since that time and was producing a high percentage of 1-septate and small spores. In some tests the irradiated plates, in contrast to the controls (fig. 2 *A*), produced a fair percentage of normal 4–5-septate spores (fig. 2 *B*) but in other experiments irradiation had little effect. Apparent-

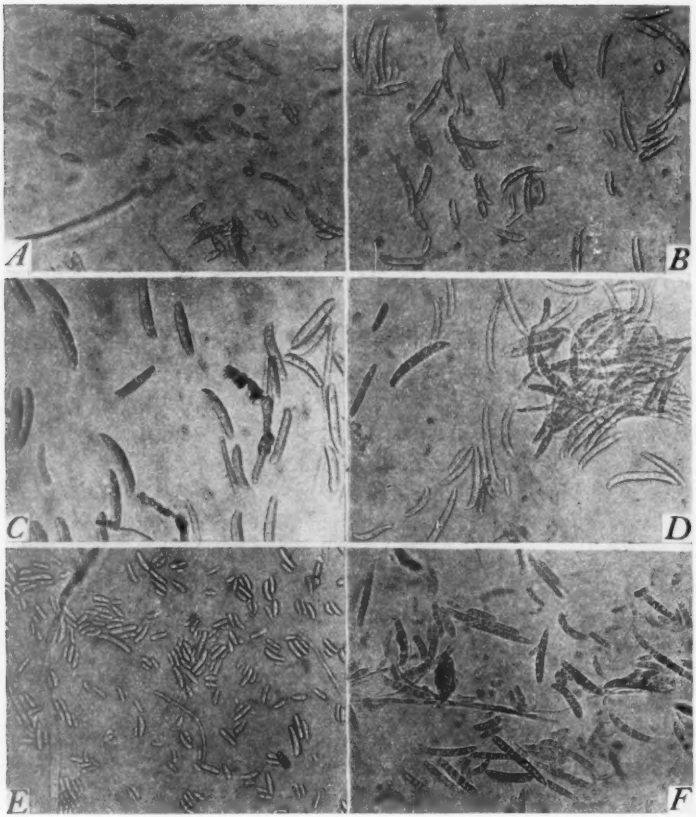


FIG. 2.—Effects of ultraviolet irradiation on cultures of *Fusarium* spp., placed 40 cm. from quartz mercury arc: A, *F. javanicum* control showing predominance of microspores; B, *F. javanicum* irradiated through vitaglass for three daily 15-minute periods with resultant increase in macrospore percentage; C, macrospores of *F. coeruleum* produced by three daily 15-minute exposures through vitaglass. D–E, *F. vasinfectum* var. *zonatum:* D, abundance of macrospores produced as result of three daily 15-minute irradiations through Corning Corex A filter 980 A (2300–6690 A.u.); E, control showing scarcity of macrospores; F, plasmolysis of protoplasm in spores as result of three daily 15-minute exposures direct to the lamp (2230–6690 A.u.).

ly macrospore production by this method cannot be predicted for *F. javanicum*.

In the case of *F. argillaceum*, the macrospore percentage on the irradiated plates was usually a little higher than on the control plates, but sometimes this did not hold true. It was found that if the mounts were made only from the new growth that occurred during the 24 hours following irradiation, the macrospore percentage was always higher and the spores normal; but if the mounts were made only from the portion of the colony that had actually been irradiated, there was considerable variation in results. The spores (fig. 1 *F*) present in this irradiated area were usually abnormal in shape, contained refractive particles apparently in part fat and glycogen, or were plasmolyzed or breaking down, so that even though present they could not be used for study of normal spores. In all experiments great care was taken to secure uniform spore mounts from all over the colony, but in the case of *F. argillaceum* this was always more difficult, owing to the toughness and irregular growth of the stunted colony after irradiation. Even those rays transmitted by vitaglass usually resulted in disruption of spores and mycelium of *F. argillaceum*, and in retarded, abnormal growth. Lengthening the period of exposure to one half-hour resulted generally in such colony stunting (measured by radial increase and amount of aerial mycelium) and death of spores that recovery did not take place in the 24 hours succeeding exposure, with the result that the macrospore percentage was less than that of the controls at the close of the experiments. Shortening exposure to one 15-minute period gave better production of macrospores. The irradiation usually resulted in the production of many chlamydospores, whereas the control plates had very few; with longer exposures the growth often was largely chlamydospores.

The most outstanding usefulness of irradiation with ultraviolet is in inducing the production of spores by strains which have not been sporulating; and the method is an advantageous one, for if the species is one responsive to ultraviolet treatment macrospores are readily obtained in a very short time. Even the production of microspores is extremely helpful in such studies, for purification of the

culture by obtaining single spore strains and for help with taxonomic identification. One strain (tentatively placed in *F. scirpi* L. and F. var. *compactum* Wr. of Section Gibbosum), isolated from decayed onion bulbs, had never produced spores in artificial culture until irradiated, when normal spores suitable for taxonomic study appeared on the experimental plates. While the treated plates had many spores (75 per cent macrospores) the control plates were still without spores of any kind. In subsequent experiments with this fungus, when the inoculum was taken from the pionnotal stage which now occurred in subcultures made from the previously treated plates, irradiation failed to bring the percentage of macrospores noticeably higher than the controls which were already producing abundant macrospores, as shown in table I.

A culture of *F. coeruleum* (Lib.) Sacc., which likewise had failed to produce spores of any kind in artificial culture, was induced to produce abundant macrospores (fig. 2 *C*) with irradiation, while the controls remained sterile. A strain of *F. sambucinum* Fuckel, which was in "abkultur," was made to produce macrospores by irradiation although the controls produced no spores; and similarly, irradiated plates of *F. culmorum* (W. G. Sm.) Sacc. produced 83.2 per cent macrospores when no conidia could be found in the control plates. In the case of the latter species it was a matter of hastened spore production, for a week later both control and treated plates showed spores. Yet even after this interval of time effects of irradiation were evident, for the control plates averaged from 40 to 56 per cent macrospores and the exposed plates from 65 to 70 per cent. DILLON WESTON (3) also found hastened sporulation in irradiated cultures of this species, but the increase in macrospore percentage here given would seem to indicate that the effect of irradiation is something more than earlier maturation. Preliminary observations of irradiated *Fusarium* spp. indicate that changes in the composition of the mycelium may be associated with the assumption of sporulation following irradiation.

It is interesting to note in table I, and in subsequent tables, that the species which responded to treatment with marked increase in macrospore production are the saprophytes and decay-producing organisms and not the vascular parasites. The only vascular para-

site which showed appreciable increase was *F. oxysporum* Schlect. forma 5 Wr. (syn. *F. oxysporum* Schlect. var. *nicotianae* Johns.), whose control plates averaged 28.3 per cent macrospores while the irradiated plates averaged 69 per cent. This culture of *F. oxysporum* f. 5 is a subculture of Dr. REINKING's culture no. R119 of *F. oxysporum* var. *nicotianae*, which is the strain represented by WOLLENWEBER in Delineata 1012 (1930 Supplement) (**30**) and described by REINKING and WOLLENWEBER in Tropical Fusaria. In the latter reference (**15**, p. 192) the authors state in text and footnote:

> *Fusarium oxysporum* var. *nicotianae* is the cause of a tobacco wilt in the United States, but the pathogenicity of the soil fungus described here has not yet been proved. . . . . Under such circumstances the fungus described here, as isolated from Honduras soil and identified as *F. oxysporum* var. *nicotianae* may touch the border line or may be even a widespread saprophytic soil form of the banana wilt *Fusarium, F. cubense.*

Incidentally the strains here tested of the banana parasite, *F. cubense* E. F. S., also obtained from REINKING, gave little response to ultraviolet. Whether or not this variation in response of two large groups of *Fusarium* species will prove constant in tests with other species of these groups, and whether such differences will prove significant in separating vascular parasites from non-vascular strains, awaits further experimentation. It is possible that more response to light might be expected from organisms which were more often exposed to light in nature on the surface of decaying material, etc., than from the vascular parasites which are spread through the soil, less exposed to sunlight, and also less dependent upon spores for their spread. At least, in contrast with the response of the vascular parasite group, irradiation was most positive in its effect on the production of macrospores in the following sections: Sporotrichiella, Gibbosum, Discolor subsection Saubinetii, Martiella, and Elegans subsection Oxysporum (except vascular parasites).

It was found, as in the previous work with *F. cepae* (**14**), that irradiation of responsive species on several days in succession was better than one longer exposure. One day's exposure with responsive species gave results, but not so many macrospores developed as were obtained by several irradiations. Fifteen minutes under vitaglass on each of three successive days was a good average period for

this purpose; under some of the filters transmitting shorter wave lengths a shorter period of time was favorable. Increasing the exposure beyond 15 minutes sometimes resulted in a still greater increase in sporulation, sometimes not. Plates of *F. decemcellulare* Brick., given three 30-minute exposures, produced 3.1 per cent macrospores as against 1 per cent after three successive 15-minute exposures and in the controls; with half-hour exposures of *F. heterosporum* Nees. var. *paspalicola* P. Henn. it was possible to get 4.5 per cent as against 1.8 per cent under 15-minute treatments. But these differences are slight and it was usually found that if treatment for 15 minutes under vitaglass was not effective, longer exposures were seldom warranted, and in some fungi, such as *F. argillaceum*, were actually lethal or detrimental to normal vegetative and reproductive development.

Multiplying the numbers of days of exposure was often more effective for conidial production than lengthening the period of a single exposure; but in species entirely unresponsive to three 15-minute treatments, even this increase in number of irradiations failed to produce spores. Table II shows the results of three and of six daily 15-minute exposures with several species of *Fusarium*. In all of the experiments shown in this table several plantings were made to the plate, and the readings made on the seventh day were from different colonies on the same plates as those read for the fourth-day results. The readings on the fourth day were, of course, made before irradiation on that day. In a few species, such as *F. vasinfectum* var. *zonatum* forma 2 and *F. oxysporum* Schlect. forma I Wr. (syn. *F. oxysporum* var. *euoxysporum* Wr.), there was noticeable increase in the six exposures, but in some of them (*F. javanicum* and *F. argillaceum*, for instance) the spore count was less at the end of the six days.

An attempt was made to see whether a cumulative effect could be obtained in some of the species which had failed to respond with macrospore production. For the purposes of this study, spores from plates which had already been irradiated were used as inoculum for new petri-dish cultures to be irradiated. The tests with *F. orthoceras* App. & Wr. will serve to illustrate the results. A strain which had been producing few spores in the stock tubes was used in the first experiments, and the usual tests involving three 15-minute

exposures resulted in a deepening of the color of the colony and the production of more microspores than the controls. Less than 1 per cent macrospores were present in controls and test plates alike. A duplicate experiment was run with three half-hour exposures which

TABLE II

EFFECTS ON SPORULATION OF FUSARIUM SPECIES OF THREE AND OF SIX DAILY 15-MINUTE EXPOSURES TO ULTRAVIOLET RAYS TRANSMITTED THROUGH VITAGLASS

SPECIES OF FUSARIUM	EXPOSED (PERCENTAGE MACROSPORES)		CONTROL (PERCENTAGE MACROSPORES)	
	3 DAYS	6 DAYS	3 DAYS	6 DAYS
F. argillaceum (Fr.) Sacc. (*W–J*)*	67.3	42.7	62.6	.....
F. bulbigenum Cke. & Mass. f. 1 Wr. (*C*)	5.4	7.0	6.7	6.0
F. conglutinans Wr. (*Wis*)	0 to −0.5	3.9	0.0	2.0
F. " " var. callistephi Beach (*C*)	7.4	3.8	1.0	1.0
F. decemcellulare Brick. (*R*)	2.2	3.1	0.6	1.0
F. heterosporum Nees. var. paspalicola (P. Henn.) Wr. (*W*)	2.0	4.5	−0.5	−0.5
F. javanicum Koord. (*R*)	39.9	9.2	17.0	17.0
F. macroceras Wr. & Rg. (*R*)	−0.5	−0.5	−0.5	−0.5
F. moniliforme Sh. var. maius Wr. & Rg. (*R*)	0.6	1.6	1.0	1.0
F. orthoceras App. & Wr. (*W*)	1.0	1.0	−0.5	−0.5
F. " var. albidoviolaceum (Dasz) Wr. (*W*)	1.7	0.9	0.0	0.0
F. oxysporum Schlect. (*R*)	−1.0	−1.0	−0.5	−0.5
F. " forma 1 Wr. (*W*)	2.2	12.7	0.0	0.0
F. " " 2 Wr. (*S*)	3.0	1.9	−1.0	1.0
F. " " 3 Wr. (*R*)	−1.0	−1.0	0.0	0.0
F. " var. aurantiacum (Lk) Wr. (*W*)	7.6	7.0	0.0	−0.5
F. redolens Wr. (*W*)	83.9	86.3	53.4	55.0
F. scirpi L. & F. var. acuminatum (E. & E.) Wr. (*R*)	64.7	99.3	78.8	79.6
F. semitectum B. & Rav. (*W*)	28.9	22.1	18.5	16.6
F. vasinfectum Atk. (*B*)	2.0	4.8	3.8	3.5
F. " strain 2 (*G*)	1.0	1.6	1.0	1.0
F. " forma 1 Wr. (*W*)	96.9	98.2	83.0	81.0
F. " v. zonatum (Sh) Wr. f. 2 Lk. & Bail. (*B*)	56.8	78.6	55.0	55.0

* Letters in italics refer to source of the culture as shown in footnote, table I.

gave results detectable from the 15-minute plates only in a slightly deeper color. Mounts of spores from the irradiated plates of these two sets of tests, and also from their controls, were then used as inoculum for petri-dish cultures, which after sufficient growth were given three daily 15-minute treatments with ultraviolet transmitted through vitaglass. Some of the previously irradiated plates and

their controls, one each of which had been used for the inoculum, were retained for comparison at the conclusion of the new tests. A control for the whole test was made by inoculating plates with mounts from the subcultures of the original unirradiated culture.

At the conclusion of the three days, the newly irradiated plates (*A*), obtained from the inoculum previously given three 30-minute exposures, showed a thick zonate light ochraceous salmon (**16**) pionnotes with many more spores than the controls, and with a deeper pionnotes than the plates from which the inoculum was taken; but there were still less than 1 per cent macrospores. The newly irradiated plates (*B*), whose inoculum came from the cultures previously given a 15-minute exposure period, also showed more spores than the control and than their inoculum plates; but there was more aerial flesh pink mycelium and a little less exposed pionnotes than on the *A* plates. Here again the microspores were more than 99 per cent of the total spore content. The newly exposed plates (*C*), with inoculum taken from the former control cultures, looked like the *B* plates and had a similar spore content. All unirradiated controls showed appressed moist or aerial white mycelium with no visible pionnotes and with a spore composition made up almost entirely of microspores.

A repetition of the experiment using plates *A*, *B*, and *C* for inoculum served to produce a slightly thicker pionnotes in all irradiated plates but no macrospore increase. The second subculture of the original unirradiated culture showed a very slight increase in spore production but no real pionnotes. Evidently the increase in spore content after irradiation was a little more than that ordinarily obtained by making subcultures from spores instead of from mycelium, for in every case treatment increased the total spore production more than did simple subculture by spore inoculum from the control. However, the tests failed entirely to produce a cumulative effect in percentage of macrospores present.

Similar results were obtained with strains of *F. conglutinans* and with *F. bulbigenum* forma 1 (*F. lycopersici*) which had also been unresponsive to early irradiation tests. In other strains of *F. bulbigenum* forma 1, which had been producing a few macrospores in stock tubes, repeated irradiation resulted in an increase in the total

spore production and in the number of macrospores present, but no appreciable increase in percentage. Thus the response seemed consistent regardless of whether the fungus treated was in "abkultur" or "hochkultur."

Results of irradiation through various filters

A distance of 40 cm. from the arc was found most advantageous for spore production. If this distance was shortened much, the factor of heat from the lamp entered in, and if it was lengthened much, the loss of the shorter rays became a factor. Although the distance from the arc and the length of exposure most favorable seemed to be about the same for most species tried, it was found that maximum production of spores was not induced in every strain when a vitaglass filter was used. This is brought out in figures 5 and 6 and in table III, which gives wave lengths transmitted by each filter and by the lamp itself. Figure 3 gives the spectrograms of the quartz mercury arc direct and through the various filters.

In the experiments with the filters, all plates were exposed for 15 minutes on each of three successive days at 40 cm. from the arc, but with the filter inserted in the lamp between the arc and the culture. During exposure the petri-dish cover was removed. Direct exposure was made without any filter in the lamp. Lids were removed from the controls also, for 15 minutes each day during exposure of the other plates. When not under the lamp, all plates were kept covered in diffuse light in the laboratory.

Although for most species tried, the vitaglass, transmitting wave lengths as short as 2650 A.u., gave maximum or very good results in spore production, maximum results with *F. vasinfectum* var. *zonatum* were always obtained by using Corex filter 980 A. (fig. 2 *D*), whose lower limit of transmission is 2300 A.u. Use of this filter gave as much as two or three times as high a percentage of normal macrospores as did the use of the vitaglass filter, although the latter filter induced three to eight times as high a percentage of macrospores as was present in the unirradiated plates (fig. 2 *E*). Use of Corex filter 986 A gave slightly better results with *F. vasinfectum* var. *zonatum* than did the vitaglass, and, although some normal spores as well as injured spores (fig. 2 *F*) occurred when the cultures were irradiated

TABLE III

EFFECT ON MACROSPORE PRODUCTION OF EXPOSING (AT 40 CM. FROM ARC FOR 15 MINUTES ON EACH OF THREE SUCCESSIVE DAYS) FUSARIUM SPECIES TO ULTRAVIOLET RAYS EMITTED BY QUARTZ MERCURY ARC THROUGH VARIOUS FILTERS

SPECIES OF FUSARIUM	CONTROL (DIFFUSE DAYLIGHT)	NOVIOL C 4880–6690 A.U.	G 586 AW 3334–3690 A.U.	FILTER NO. 1 3020–6690 A.U.	VITAGLASS 2650–6690 A.U.	COREX 086 A 2535–4340 A.U.	COREX 080 A 2300–6690 A.U.	DIRECT. 2230–6690 A.U
F. decemcellulare Brick. (*R*)† (section Spicarioides)	Macro. rare (8 mm.)‡	Macro. rare (9)	Macro. rare (8)	Macro. less than ½% * (8)	Macro. ½% * (8)	Macro. less than ½% * (6.5)	Less than ½% * (5.5)	No macro.; few micro. (4-under agar surface)
F. nivale (Fr.) Ces. (*D–L*) (s. Arachnites)	59%§ (10)	61.3% (10)	63.5% (10)	61.8% (10)	75.2% * (10)	73.1% * (10)	69% * (10)	76.7% (5)
F. sporotrichioides Sh. (*B*) (s. Sporotrichiella)	10.9% (15.5)	10.5% (17)	9.6% (16.5)	11.8% (16)	51.7% * (16)	53.5% * (13.5)	34.3% * (12.5)	16% (7.5)
F. semitectum B. & Rav. (*R*) (s. Arthrosporiella)	18.4% (15)	18.9% (16)	18.1% (15)	16.8% * (15)	18.8% * (15)	20% * (12)	25.3% * (13)	39.5% (?) (3-on surface 12-beneath)
F. scirpi L. & F. var. acuminatum (E. & E.) Wr. (*R*) (s. Gibbosum)	51.9% (17.5)	55% (22.5)	64.6% (17)	62.2% (18.5)	85.7% * (17)	71.1% * (12)	71.9% * (12)	Macro. & micro. rare (2)
F. sambucinum Fuckel (*B*) (s. Discolor)	No spores (8)	No spores (7)	No spores (8)	Spores rare, mostly macro. * (7)	95% * (7)	No spores (4)	No spores (5)	No spores (1)

* Total sporulation (macrospores and microspores) greater than controls.
† Letters in italics refer to source of culture as given in footnote, table I.
‡ Figures in parentheses express radial increase in mm. during 3 days following first exposure.
§ All percentages express percentage of macrospores present.

TABLE III—*Continued*

F. neoceras Wr. & Rg. (*R*) (s. Liseola)	1.9%	2.5%	2.5%	6.3% *	22.7% *	8.6% *	4.4% *	Macro. & micro. rare
	(14.5)	(14)	(15)	(14.5)	(14)	(14)	(14)	(11)
F. orthoceras App. & Wr. (*W*) (s. Elegans s.s. Orthocera)	No macro.; very few microspores	Like control	Like control	No macro.; thin pionnotes *	Less than 1%; * thin pionnotes	Rare; * thin pionnotes	Like 986 A *	All spores rare
	(14)	(14)	(14)	(15)	(14)	(12)	(8.5)	(6.5)
F. conglutinans Wr. (*Wis*) (Elegans-Orthocera)	No macro.	No macro.	Macro. rare	Macro. rare *	Less than 1% *	Rare *	No macro. *	No macro.; very few micro.
	(10)	(10)	(10)	(10)	(10)	(9)	(9)	(5)
F. bulbigenum Cke. & Mass. (*W*) (Elegans-Constrictum)	3.3%	1.7%	3.3%	2.7%	8.1% *	17.2% *	11.4% *	12.8% (6-mostly beneath agar)
	(14.3)	(15)	(15)	(15)	(13)	(12)	(11)	
F. vasinfectum Atk. var. zonatum (Sh) Wr. (*W*) (Elegans-Oxysporum)	5.6%	5.1%	5.6%	10.9%	17.5% *	19.2% *	37.7% *	17.6% (8.2-mostly under agar)
	(13.3)	(13.5)	(13.3)	(14)	(14.4)	(12)	(13)	
F. javanicum Koord. (*R*) (s. Martiella)	3.4%	4.2%	5.6%	6% *	10.2% *	9.2% *	8.6% *	Spores rare; many shattered (2 above agar 5 mm. total)
	(16)	(15.5)	(17)	(15.5)	(14)	(10)	(8)	
F. javanicum Koord. var. theobromae (App. & Strk.) Wr. (*R*) (s. Martiella)	Macro. rare	Macro. rare	Macro. rare	Macro. rare	Less than 1% *	Less than 1% *	More than vitag. but not 1% *	Like 980 A but spores abnormal
	(17)	(16)	(16)	(17)	(12)	(8)	(9)	(3)
(F. coeruleum (Lib) Sacc. (*S*) (s. Martiella)	No spores	No spores	No spores	Few spores * 95.6%	Many spores * 99.4%	Fewer spores than vitag. * 91.9%	Like 986 A * 85.6%	No spores; mycelium empty or granular
	(6)	(5.5)	(6)	(5)	(5)	(3)	(3.5)	(1)

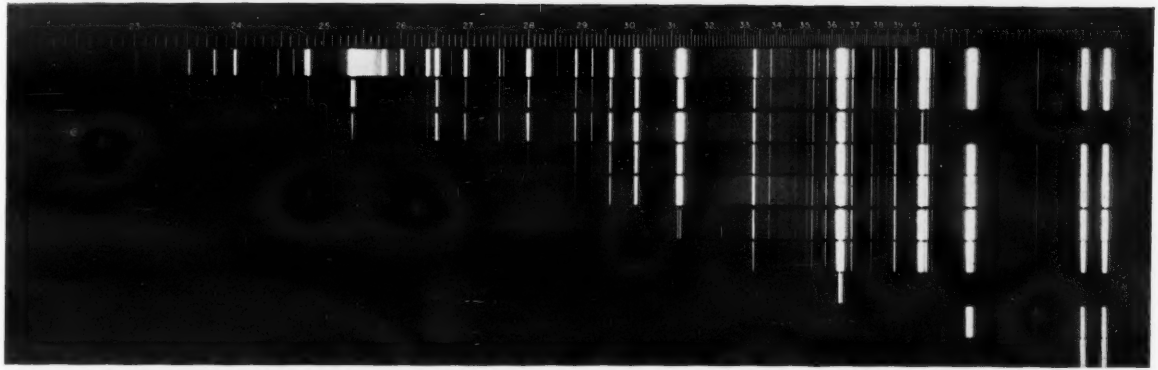


FIG. 3.—Spectrograms of quartz mercury arc direct and through various filters: A, direct arc (2230–6690 A.u.); B, Corning Corex A (980 A), (2300–6690 A.u.); C, Corex red-purple (986 A), 2535–4340 A.u.; D, vitaglass (2650–6690 A.u.); E, petri dish (2800–6690 A.u.); F, glass filter no. 1 (3020–6690 A.u.); G, glass filter no. 6 (3120–6690 A.u.); H, Corning (G 586 AW), (3334–3690 A.u.); I, Noviol A (4340–6690 A.u.); J, Noviol C (4880–6690 A.u.). Some faint lines showing lowest limits of transmission are lost in reproduction.

directly, usually the percentage was less than with any of the three filters transmitting as low as 2650 A.u.

The peak of macrospore production for *F. sporotrichioides* occurred consistently with the use of Corex filter 986 A (fig. 4 *A*), with which the shortest wave length transmitted was 2535 A.u. Although there was little difference between the results with this filter and those with vitaglass, the small advantage in number of macrospores produced was always on the side of the Corex 986 A (table III). Fewer sporotrichial, pyriform conidia (**19,** p. 183) occurred with 986 A than with vitaglass. When the fungus was exposed to the still shorter wave lengths transmitted by Corex filter 980 A, there was a decided drop in macrospore production but also in the sporotrichial conidia, the microspores present being ovate rather than pyriform. With filters transmitting no shorter waves than 3020 A.u. there was no noticeable change from the control plates, all of which produced few macrospores and a high percentage of pyriform conidia (fig. 4 *B*). *F. bulbigenum* Cke. and Mass. ran a much more evident peak under Corex filter 986 A than did *F. sporotrichioides*, for in every experiment the spore production here was much better and the macrospores produced (fig. 4 *C*) were of better shape than under vitaglass (fig. 4 *D*) and Corex 980 A. Sporulation on the control plates was almost entirely microspore (fig. 4 *E*).

In every instance, however, the fungi which developed maximum macrospore production under a filter transmitting shorter waves than 2650 A.u. gave increased sporulation with vitaglass also. Therefore this filter seems to be the most advantageous one to use for this purpose. The tests with *F. semitectum* B. & Rav., which were summarized for table III, show no increase in macrospore percentage under vitaglass, but in most individual experiments there was some increase, as is shown by the average in table I.

In the tests with *F. bulbigenum* another result is shown in the percentages cited in both table III and figure 5 for exposure directly to the arc without filters. It will be noted that the average percentage for direct exposure is slightly greater than that for Corex 980 A, making an irregularity in the curve. This irregularity was consistent for all individual experiments with *F. bulbigenum*, as well as for the averages, and was no doubt due to the lethal effects of

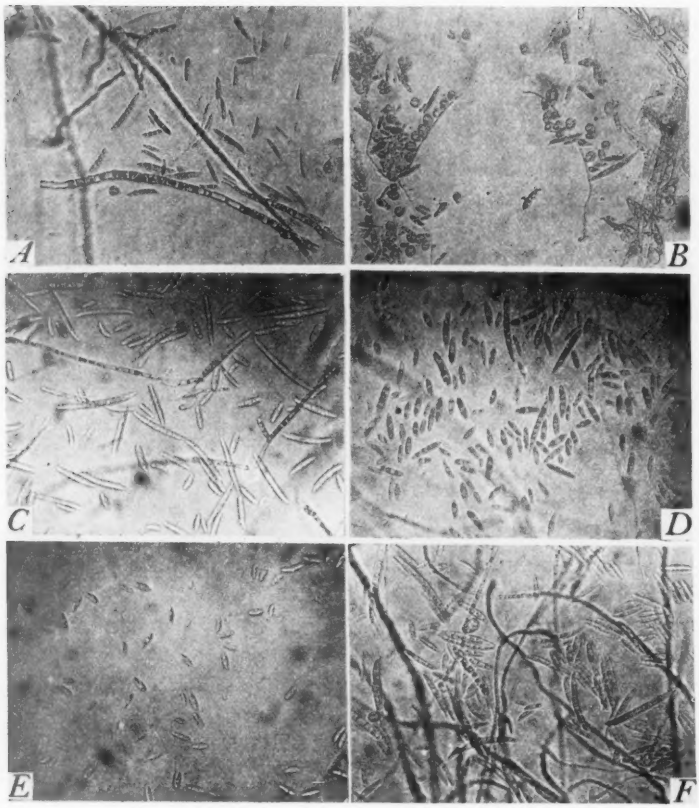


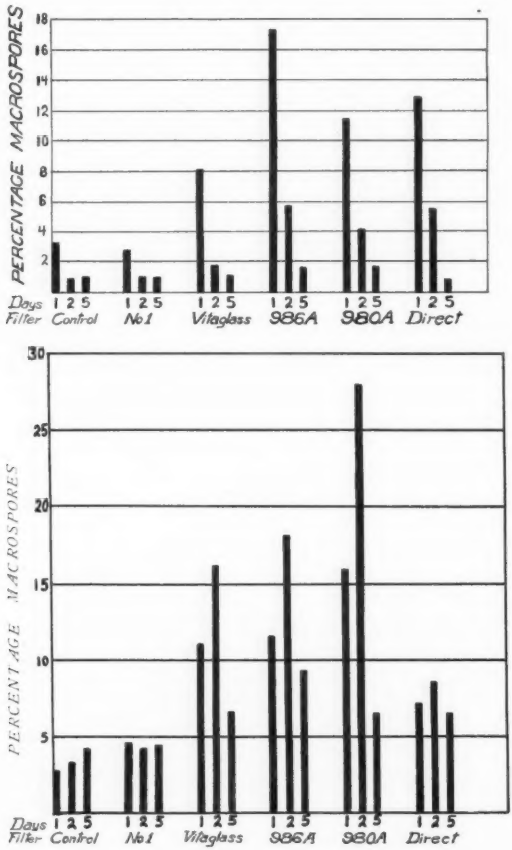
FIG. 4.—Effects of ultraviolet irradiation on cultures of *F. bulbigenum* and *F. sporotrichioides* placed 40 cm. from quartz mercury arc: A, increase in macrospores and decrease in sporotrichiate conidia in culture of *F. sporotrichioides* given three daily 15-minute exposures to Corex red-purple filter 986 A; B, control of *F. sporotrichioides* showing scarcity of macrospores and predominance of sporotrichiate conidia; C, abundance of macrospores of *F. bulbigenum* 24 hours after third daily 15-minute exposure through Corex red-purple filter 986 A; D, spores of *F. bulbigenum* present after three daily 15-minute exposures through vitaglass (2650–6690 A.u.); E, control of *F. bulbigenum* showing absence of macrospores; F, increase in microspore content of *F. bulbigenum* 48 hours after third daily 15-minute exposure through Corex red-purple filter 986 A (2535–4340 A.u.).

the shorter waves transmitted. In all plates exposed directly to the arc, this fungus showed a high percentage of spores whose contents were broken up into globules or which were apparently empty of protoplasm. Mycelium also exhibited a similar condition, but spores seemed to break up more readily and many spores were disrupted or badly shattered. Sometimes it was possible to recognize, for counting purposes, a macrospore which was disrupted or shattered, but shattered microspores could not be so easily detected. Often great masses of loose material or small clumps of floating glycogen and fat particles were suspected of coming from shattered spores, but could not, of course, be interpreted for counting. This decrease in detectable microspores could easily account for the upward trend of macrospore percentage when the plates were exposed directly to the arc.

Such a result occurred also with *F. nivale*, and with *F. semitectum*, which really had its maximum production of macrospores under Corex 980 A but which would seem from the average percentages shown in table III to be most favored by direct exposure. In reality *F. semitectum* was even more subject to destruction by the direct rays than was *F. bulbigenum*. The colony was much stunted and the spores badly shattered, again making a representative count impossible. Under Corex 980 A, on the other hand, the spores were normal.

All of the *Fusarium* species tried were injured somewhat by direct exposure to the arc: decided colony stunting, as measured by radial growth (figs. 7, 9, 10, 13); plasmolysis of protoplasts in mycelium and spores (fig. 2 *F*); empty and abnormally shaped cells were noted. In some cells the protoplasm was present as a single comparatively uniform mass; in others numerous small refractive globules appeared. Microchemical reactions indicated the presence of glycogen and fat globules, some of which stained with Sudan III, others of which were partially saponified by sodium hydroxide. However, the severity of injury varied greatly with the species. *F. argillaceum* was extremely susceptible to injury by the shorter waves, those from the direct arc often causing the mycelium to go over almost entirely into production of chlamydospores, and stunt-

ing occurring even when the shortest waves were screened out by vitaglass. In some species there was little spore shattering under full transmission by the lamp, but spore production was checked so



FIGS. 5–6.—Fig. 5 (above), percentage of macrospores of *F. bulbigenum* present at end of one, two, and five days following irradiation with ultraviolet transmitted directly from quartz mercury arc and through various filters. Fig. 6 (below), percentage of macrospores of *F. vasinfectum* var. *zonatum* present at end of one, two, and five days following irradiation with ultraviolet transmitted directly from quartz mercury arc and through various filters.

that the actual number of spores present was lessened, and sometimes no spores occurred (as in *F. coeruleum*) or they were very rare (as in *F. scirpi* var. *acuminatum*). In other species, as in *F. javanicum* and *F. javanicum* Koord. var. *theobromae* (App. & Strk.) Wr., spores were produced between irradiations but were so shattered and abnormal after direct irradiation as to make representative spore count impossible. In no other case was injury as marked under vitaglass as was usually true in the case of *F. argillaceum* (fig. 1 *F*), although in some forms decrease in growth rate did occur with this filter, as is shown by the figures in table III expressing radial increase in millimeters during the three days following first exposures and by figure 8. Although decrease in growth rate sometimes occurred with this filter and often occurred with the two filters transmitting shorter waves (figs. 9, 10), only in rare cases was there any injury to spores and mycelium such as is described here for direct exposure.

In a number of species the total sporulation was somewhat augmented when irradiations were made through filter no. 1, which transmitted to 3020 A.u., and in some the percentage of macrospores was greater. The macrospore percentage of *F. neoceras* was tripled with filter no. 1 and that of *F. vasinfectum* var. *zonatum* sometimes doubled, but for the most part slight results were obtained with filters transmitting no lower than 3020 A.u. Increasing the length of exposure with this filter gave greater macrospore production with *Fusarium* spp., thus corroborating the findings of RAMSEY and BAILEY (**14**) for *F. cepae* and *Macrosporium*. The total sporulation produced under the three filters transmitting lower than 3020 A.u. was almost invariably greater than on unirradiated plates. Experiments with the Corex D filter in the Stewart health-lamp (wave lengths transmitted given under "Methods") gave essentially the same results as vitaglass. The following percentages of macrospores were obtained for the species named, in irradiated and non-irradiated plates respectively: *F. sporotrichioides*, given three daily 15-minute exposures, — macrospores 56.3%, control 36.5%; *F. semitectum*, exposed, — macrospores 6.5%, control 3.5%; *F. moniliforme* Sheld. var. *subglutinans* Wr. & Rg., exposed, — macrospores 7.2%, controls 4.6%; *F. orthoceras*, exposed, macrospores 1.2%, control (no macrospores); *F. vasinfectum* var. *zonatum*, exposed, — macro-

spores 46.7%, control 5%; and *F. argillàceum*, exposed, — macrospores 23%, control 22%.

In some species, as in *F. culmorum* previously mentioned, the results of irradiation were evident a week after irradiation, but in many species the effect was lost soon after the experiments were concluded. The germination of macrospores resulting in the production of many microconidia accounts for most of this change. In *F. bulbigenum* the drop in macrospore percentage for this reason became pronounced on the second day after irradiation ceased (figs. 4 *F*, 5), and by the fifth day all plates were essentially reduced to the same macrospore percentage as in the control plates. Consequently it was imperative for accurate comparison that all spore counts be made as near as possible to the end of the 24-hour period following irradiation.

In some of the other species the maximum production of macrospores was evident on the second day following irradiation, as in the case of *F. vasinfectum* var. *zonatum* (fig. 6). On the third day the percentage began to drop, and by the fifth day the percentages were nearing those of the controls. Usually the rise in macrospore count on the directly irradiated plates of *F. vasinfectum* var. *zonatum* was especially noticeable, although in the experiments averaged in figure 6 the increase is not striking.

Effect of ultraviolet on Fusarium species with proven perfect stage

Some tests were made with those species of *Fusarium* with proven perfect stages, to see whether irradiation with ultraviolet would induce perithecial formation. The results of these experiments are summarized in table IV and need little discussion. The 4-day old cultures were treated as in the previous tests with three 15-minute exposures at 40 cm. from the mercury arc, the rays passing through the vitaglass filter. Essentially the same results were obtained whether the filter was in the lamp or used as a petri-dish cover. A duplicate set of tests was run at the same time, however, in which the cultures were exposed to the direct rays from the mercury arc for 4 seconds at a distance of 21 cm. from the arc. This latter set was run since STEVENS (**20**) obtained many perithecia of *Glomerella*

cingulata (Stoneman) Sp. & Schrk. by exposing them to direct rays for 4 seconds at 21 cm.

While the results on conidia production were essentially as one would expect from previous experiments, no perithecial development was evident at the conclusion of the experiments. A few tests with 1 second and 5 seconds direct and with 15-minute exposures under the various filters were also negative. STEVENS found the rudiments of perithecia of *Glomerella cingulata* 15 hours after irradiation and obtained mature ascospores in 4 days. In the case of *Colletotrichum lagenarium*, although the plexi began to develop 24 hours or less after irradiation and the perithecia 1–10 days later, he found that sometimes the perithecia did not appear for 23–30 days. Therefore the species of *Fusarium* here tested were held for several weeks to two months. Only in the case of a subculture of REINKING'S strain 129 (**33**, p. 168) of *F. javanicum* Koord. var. *theobromae* (App. & Strk.) Wr. did perithecia develop at all, and then only in one experiment. In the work with two strains of this fungus the mature perithecia of the perfect stage, *Hypomyces ipomoeae* (Hals.) Wr., were found seven weeks after irradiation on two plates of one strain which had been thrice irradiated for 15 minutes; and vestigial perithecia were found in one of the second-strain cultures which had been irradiated directly for 4 seconds on each of three successive days. Other plates similarly treated bore no perithecia, and those found on the plates mentioned occurred only on the glass sides of the petri dishes at the edge of the colony, in the new growth since the irradiation; therefore although controls were also negative there was no real evidence that ultraviolet rays were responsible for the occurrence of the few scattered perithecia which appeared in one test.

In another strain of *Hypomyces ipomoeae*, which was producing perithecia in artificial culture, similar tests brought no increase in perithecial production over the control plates which also produced mature perithecia. It may be worthy of note that growing WINELAND'S two strains (**27**) of *F. moniliforme* together under ultraviolet radiation did nothing to restore their erratic tendency to produce the perfect stage, *Gibberella moniliformis* (Sh.) Wineland. These subcultures were not, however, producing the "blue-black globoid

TABLE IV

SUMMARY OF EXPERIMENTS SHOWING EFFECTS OF ULTRAVIOLET ON SPECIES OF FUSARIUM WITH KNOWN PERFECT STAGE

SPECIES OF FUSARIUM	PERFECT STAGE	RESULTS OF IRRADIATION		CONTROL
		4 SEC. DIRECT, 21 CM. FROM ARC	15 MIN. THROUGH VITAGLASS, 40 CM. FROM ARC	
F. decemcellulare Brick. (*R*)* (s. Spicarioides)	Calonectria rigidiuscula (B. & B.) Sacc.	No colony stunting; no perithecia, no macrospores; fewer microspores than control	No colony stunting; no perithecia, no macrospores; more micro. than control	No perithecia; only 0-septate spores
F. nivale (Fr.) Ces. (*D–L*) (s. Arachnites)	Calonectria graminicola (B. & B.) Wr.	No colony stunting; no perithecia; 70.5% macro.; deeper color than control	No colony stunting; no perithecia; 84.8% macro.; deeper color than control	Few spores; no perithecia 35.5% to 60.2% macrospores
F. equiseti (Cda) Sacc. var. bullatum (Sherb.) Wr. (*R*) (s. Gibbosum)	Gibberella intricans Wr.	No stunting; no perithecia even when held several weeks; no macrospores	Same as 4″ direct	No perithecia; no macrospores
F. sambucinum Fuckel (1 strain from *S* and one from potato. Results identical)	Gibberella pulicaris (Fr.) Sacc.	Much stunting; no perithecia even when held 2 months; spores none to rare macrospores	Much stunting; no perithecia in 2 months; spores few to moderately plentiful; 100% macro.	No perithecia in 2 months; no spores
F. graminearum Schwabe (2 strains *W–L*) (s. Discolor Saubinetii)	Gibberella saubinetii (Mont.) Sacc.	Slight stunting; no perithecia in several weeks; 100% macrospores	No stunting; otherwise as for direct	No perithecia; 100% macrospores
F. lateritium Nees. var. mori Desm. (*W*) (Syn. F. urticearum (Cda) Sacc.) (s. Lateritium)	Gibberella baccata (Wallr.) var. moricola (Ntrs) Wr.	No stunting; total sporulation greater than control; 1.3% macrospores	2% macrospores; otherwise as direct; no perithecia in either	Less than 1% macrospores; no perithecia

* Letters in italics refer to source of culture as given in footnote, table I.

TABLE IV—*Continued*

F. moniliforme Sheldon (Wineland's Y 29) (*J*)	Gibberella moniliformis (Sh.) Winel.	No stunting; no perithecia in 2 months; more spores than control but macrospores rare	No stunting; no perithecia in 2 months; more spores than direct; 1% macrospores	No perithecia in 2 months; macrospores rare
Wineland's Y 15 (*J*)	Gibberella moniliformis (Sh.) Winel.	Slight stunting; no perithecia in 2 months; occasional macrospore; fewer than with vitaglass	Slight stunting; no perithecia in 2 months; less than 1% macrospores	No perithecia in 2 months; macrospores rare
Y 29 & Y 15 on same plate		No perithecia in 2 months	As for direct	No perithecia
F. moniliforme Sheld. var. maius Wr. & Rg. (*R*) (s. Liseola)	Gibberella fujikuroi (Saw.) Wr.	Slight stunting; no perithecia in 2 months; more spores than control; less than 1% macrospores	As for direct except thicker pionnotes and 2.1% macrospores	No perithecia in 2 months; macrospores rare
		At conclusion of irradiation period		
F. javanicum Koord. var. theobromae (App. & Strk.) Wr. (*R*)	Hypomyces ipomoeae (Hals) Wr.	No stunting; no macrospores; no perithecia; fewer micro. than control	Slight stunting; few macrospores; no perithecia	No macrospores; no perithecia
		7 weeks after irradiation period		
		A few macrospores; no perithecia	A few macrospores; perithecia at edge of colony on side of petri dish only	A few macrospores; no perithecia
		At conclusion of irradiation period		
F. javanicum Koord. var. theobromae (App. & Strk.) Wr. (*R*) 2nd strain from (*R*) labeled	"Hypomyces ipomoeae" but bearing no perithecia on receipt	Slight stunting; no macrospores; no perithecia	Slight stunting; macrospores less than 1%; no perithecia	No macrospores; no perithecia

TABLE IV—*Continued*

Species of Fusarium	Perfect stage	Results of irradiation		Control
		4 sec. direct, 21 cm. from arc	15 min. through vitaglass, 40 cm. from arc	
		7 weeks after irradiation period		
		A few macrospores and a few vestigial perithecia on edge of petri dish only	A few macrospores; no perithecia	
F. javanicum Koord. var. radicicola Wr. (*R*)	Hypomyces ipomoeae (Hals.) Wr. f. 1 Wr.	No stunting; 2.1% macrospores; no perithecia even after 2 months	No stunting; 5.3% macrospores; no perithecia in 2 months	Less than 1% macrospores; fewer micro. than on exposed plates; no perithecia
F. solani (Mart. pr. p) App. & Wr. var. eumartii (Carp.) Wr. (*S*)	Hypomyces haematococcus (B. & Br.) Wr.	Some stunting; microspores only; no perithecia in 2 months	Slight stunting; microspores only; no perithecia in 2 months	Microspores only; no perithecia in 2 months
F. solani App. & Wr. var. minus Wr. (*R*)	Hypomyces haematococcus var. breviconus Wr.	No stunting; no macrospores; no perithecia in 2 months	No stunting; less than 1% macrospores; no perithecia in 2 months	Microspores only; no perithecia in 2 months
F. solani App. & Wr. var. striatum (Sherb.) Wr. (s. Martiella)	Hypomyces haematococcus var. cancri (Rutg.) Wr.	No stunting; only microspores; no perithecia in 2 months	As for direct	Microspores only; no perithecia in 2 months
F. argillaceum (Fr.) Sacc. (*W*) (s. Ventricosum)	Hypomyces solani Rke. & Berth.	Colony very stunted; macrospores none to rare; no perithecia in 3 months; many chlamydospores	Colony stunted; spores few but mostly macrospores (60–70%); no perithecia in 3 months; many chlamydospores	Spores usually more plentiful in control but 20–40% macrospores; no perithecia in 3 months; chlamydospores rare

bodies" which she found present when they were in good condition for pairing and resultant production of perithecia.

Decrease in growth rate and sporulation

Although there was in practically every instance much colony stunting owing to decrease in growth rate and lethal effects of the shorter rays when the cultures received the full emanations from the lamp, the amount of stunting which occurred with the several filters was variable, as is shown in tables III and IV. The stunting following direct exposure was always accompanied by lethal effects, such as plasmolysis of cells in the mycelium or spore destruction; but only with certain species were such conditions evident upon microscopic examinations of the cultures exposed to rays passing through the filters. The decrease in growth rate, as measured by the radial increase in millimeters during the three days following the first exposure, was usually very slight or lacking when vitaglass was used, and usually greater when the Corex filters 986 A and 980 A were used; but this varied for the species irradiated. It will be noted from table III that *F. nivale* and *F. neoceras* usually showed no stunting except upon direct exposure, but occasionally in the same experiment one plate would show stunting under a given filter and the others not (fig. 12). This was not correlated with variations in percentage of macrospores. Thus not only was there variation among the different organisms in this matter but sometimes the same strain reacted differently. *F. decemcellulare* sometimes showed slightly decreased growth rate under vitaglass, usually not; *F. javanicum* (fig. 11) and *F. javanicum* var. *theobromae* (fig. 7) usually showed some stunting when this filter was used, but sometimes not. *F. vasinfectum* var. *zonatum* usually showed no stunting with vitaglass and often none with the two filters transmitting shorter wave lengths; but sometimes there was slight stunting of the colonies under these last filters (fig. 13). There was always stunting with direct exposure, and usually the radial increase after direct irradiation was begun was largely beneath the surface of the agar, the agar screening out the detrimental shorter waves. This was also true of a great many of the other species. For example, the radial increase during the three days following the first direct exposure of *F. semitectum* was 12 mm.

beneath the surface of the agar and only 3 mm. on the surface, while that of the controls was 15 mm. on the surface. In *F. coeruleum* there was almost no growth above the surface on direct plates (fig. 9; table III). In every experiment normal growth was resumed from sub-agar mycelium or aerial mycelium a day or two after cessation of irradiation.

Although decrease in growth rate and macrospore production were often associated, there were so many exceptions that there seemed to be no definite correlation between the production of macrospores after irradiation and inhibition of growth. Therefore, the results of irradiating many species of *Fusarium* would seem to corroborate the findings of RAMSEY and BAILEY (**14**) for *Macrosporium* and for *F. cepae*, that increased spore production subsequent to irradiation with ultraviolet is not a direct result of inhibition of growth rate. Surely inhibition of growth rate is not alone responsible, for decreasing the rate of growth by exposure to higher or lower temperatures or by using less favorable media did not produce the same results. Through personal correspondence with Miss STRONG and G. H. COONS, the writer learned that in no case did abundant sporulation accompany the inhibition in growth rate of *Fusarium* species owing to the toxic effect of dyes (**2**).

HUTCHINSON (**6**), in his work with the response of *Colletotrichum phomoides* to ultraviolet, found similar lack of correlation between the growth rate and sporulation. With full mercury arc irradiation he always got a retardation of growth. However, this was accompanied by increased sporulation only under exposures of 15 seconds to 2 minutes, whereas sporulation decreased when exposures were for more than 2 minutes. He considered this as an indication that sporulation is an inverse expression of growth rate only within certain limits, and that there is an optimum amount of irradiation causing sporulation. He states that certain monochromatic bands retarded growth and stimulated production of acervuli while with other wave lengths there was initial retardation of growth followed by stimulation and also hastening of sporulation. Certain waves constantly stimulated growth and had no apparent effect upon the time of development of spores; but on the other hand wave lengths

4078 A.u. and 5819 A.u., which produced the most marked stimulation of growth, also caused early development of acervuli. Therefore he concluded that retardation and extreme stimulation of growth are accompanied by early sporulation, while irradiations causing intermediate stimulation of growth have no apparent effect in the development of acervuli.

In the present work with *Fusarium*, some species, such as *F. moniliforme*, *F. bulbigenum* forma 1, etc., which showed a decrease in the size of the colony when irradiated, showed no increase in macrospore production. On the other hand, the radial increases given in table III show that macrospore production was greatly increased when *F. nivale* (fig. 1 *C*), *F. sporotrichioides* (fig. 14), *F. scirpi* var. *acuminatum*, *F. neoceras* (fig. 12), and *F. vasinfectum* var. *zonatum* (fig. 13) were subjected to ultraviolet radiation through vitaglass, even when there was no decrease in the rate of growth during this treatment.

Irrespective of whether inhibition of growth of *F. vasinfectum* var. *zonatum* took place under filters 980 A and 986 A, an increased percentage of normal macrospores always occurred. In contrast, *F. javanicum* var. *theobromae* (fig. 7), which showed much stunting under these two filters, showed very little spore increase; in fact, the vitaglass plates, which showed decrease in growth rate but no microscopic evidence of spore destruction, often showed less total sporulation than the control plates. A culture of *F. sambucinum*, treated for 4 seconds direct on three successive days, grew only 3 mm. during those three days; whereas the controls averaged 13-mm. growth but neither set of plates showed any spores; yet duplicate cultures given three 15-minute exposures to rays no shorter than 2650 A.u. likewise grew only 3 mm. and showed considerable spore production. *F. equiseti* var. *bullatum* sometimes exhibited decreased growth after 15-minute irradiation periods, sometimes not; but in neither case did irradiation increase macrospore production. And in the one experiment where there was considerable decrease in colony size, the control plates averaging 48 mm. diameter at the conclusion of the experiment and the plates that received 15-minute irradiation with waves of 2650 A.u. and above averaging 28 mm.

diameter, the control plates showed a greater total spore production than the exposed ones, and rare macrospores as against no macrospores on the treated plates.

These data and the fact that the acme of spore production, except in a few species, occurs with the vitaglass filter which gives no detectable decrease or, at least, the smallest decrease in growth rate (as measured by radial increase of the colony and by observation of amount of aerial mycelium present), would seem to indicate that the increase in macrospores was the result of stimulation effected by ultraviolet rays rather than an indirect result of the inhibition in growth. Further apparent evidence for this conclusion lies in the fact that if a fungus were one responsive to treatment with ultraviolet, old petri-dish cultures of it, which had completely filled the plate and so were arrested in growth, could be made by irradiation to produce spores even on the old mycelium.

What changes take place in the mycelium subsequent to irradiation and immediately preceding sporulation have not yet been worked out. Tests for change in pH of mycelium or of medium as a result of irradiation were negative. No tests were made for the presence of hydrogen peroxide, although it is known that some workers consider the lethal action of ultraviolet to be an indirect one due to development of hydrogen peroxide in the nutrient substrate exposed to the rays. COONS (1) found that he could use a dilute solution of hydrogen peroxide to replace the light stimulus in the production of fruit bodies of *Plenodomus fuscomaculans*. He states that the action of light was modified and controlled by the condition of the mycelium, which is a resultant of all the acting environmental factors. Macroscopically undetectable differences in mycelium condition may also account for the variation in reaction of *Fusarium* species to ultraviolet, and it is thought that microchemical studies of the mycelium previous to and subsequent to irradiation of responsive species may throw considerable light on this question.

No work was done with intensity of the ultraviolet rays involved, since the object of this study was primarily to determine the usefulness of ultraviolet radiation in taxonomic studies of *Fusarium*. RAMSEY and BAILEY had found in the work on *F. cepae* and *Macrosporium* that, while intensity was an important factor, it apparently

is not primarily responsible for the variation between the different filters in amount of increase in sporulation. SWANN and DEL ROSARIO (**26**), working with *Euglena*, found that the predominance of the lethal effect of one monochromatic ultraviolet line over that of another given line "is not explicable entirely as a matter of absorption, but involves, in addition, other characteristics of the wave lengths concerned."

Discussion

While the chief value of ultraviolet irradiation of *Fusarium* possibly lies in inducing the production of macrospores of certain species when the macroconidia are lacking in the strains under observation, there is also some promise that ultraviolet radiation may be useful for further verification of systematic identification of some species. The consistency of response to ultraviolet of certain forms which characteristically produce macrospores or fail to produce macrospores upon irradiation seems of some diagnostic value. It is interesting to note in this respect that of the ten strains of *F. moniliforme* and three authentic varieties exposed to ultraviolet treatment, none gave noticeable increase in percentage of macrospores; while *F. neoceras* (**33**), which is the outstanding species morphologically in section Liseola, also stands definitely apart from the other forms of the same section in its response to ultraviolet by abundant macrospore production. *F. argillaceum* also, which stands alone as the only species in its section, is the only species tested which was greatly injured by the rays passing through vitaglass and which showed the striking tendency to go over into chlamydospore production as a result of irradiation. The contrast in response between vascular parasites and other species in section Elegans has already been discussed.

Variation in reaction to the various filters is scarcely sufficient to be of help in separating species, but the responsiveness or nonresponsiveness to macrospore production and the variation in colors intensified by exposure to ultraviolet might easily be of some help in classification. In colored *Fusarium* species, such as *F. bulbigenum* (fig. 8) and *F. coeruleum* (fig. 9), the colors became increasingly intense with filters transmitting lower wave lengths. In the case of some species whose mycelium was white in culture tubes, colors

developed under irradiation by the shorter rays. The mycelium of *F. javanicum*, for instance, which was white under filters transmitting no lower than vitaglass (fig. 11), became pale violet grey to pale glaucous blue above a white to pearl grey background when irradiated through filter 986 A, deepened to deep glaucous grey under filter 980 A, and was dusky green blue (2) to green blue slate when irradiated direct (fig. 10). It therefore stood out in marked contrast to its variety, *F. javanicum* var. *theobromae*, which remained white even when irradiated directly (fig. 7).

It is felt that while the use of response to ultraviolet radiation as an aid in differentiation of *Fusarium* species is of less value than morphological and pathogenicity studies, it may add one more physiological test in instances of difficult diagnosis. The value of irradiation for inducing macrospore production in short time in many species is beyond question. It must be realized, however, that it cannot always be applied, for there are many cases in which it fails to induce macrospore production.

Summary

1. In most of the 59 species, varieties, and forms of *Fusarium* exposed to the ultraviolet rays transmitted by a Cooper-Hewitt quartz mercury-arc lamp through filters transmitting waves as low as 2650 but no lower than 2300 A.u., there was an increase in total sporulation (macrospores and microspores) if not in the percentage of macrospores present. This increase was usually evidenced by the production of a pionnotes or of sporodochia; or at least by greater intensity of mycelium, stroma, and spore mass colors. Colors not present in the control developed in the mycelium of some forms when exposed to the shorter rays of ultraviolet.

2. In some species there was a slight increase in spore production with filters transmitting no lower than 3020 A.u., but with filters transmitting no ultraviolet results were negative.

3. Some species, such as *F. neoceras*, *F. cepae*, *F. solani* var. *medium*, *F. sporotrichioides*, *F. vasinfectum* var. *zonatum* and forma 1 and 2, *F. bulbigenum*, and *F. redolens*, always gave very striking increase in macrospore percentage with three daily 15-minute treatments under vitaglass (transmitting to 2650 A.u.).

4. Some other species gave favorable although not such striking results in macrospore increase.

5. Many species, such as *F. conglutinans*, *F. bulbigenum* forma 1, *F. moniliforme* and its varieties, *F. orthoceras* and its varieties, *F. oxysporum*, etc., showed very little or no macrospore increase after irradiation.

6. Except in the cases of *F. scirpi* var. *acuminatum*, *F. javanicum*, and *F. argillaceum*, the tendency to respond or not to respond to irradiation with macrospore production was consistent and seemed characteristic of the species or variety.

7. All of the species which responded to the treatment, except *F. oxysporum* var. *nicotianae*, were saprophytes or decay producers. The culture of *F. oxysporum* var. *nicotianae* was one which had not been tested for pathogenicity.

8. All of the known vascular parasites which were tested failed to show appreciable macrospore increase as a result of irradiation.

9. The most extensive spore production induced by irradiation occurred in species of sections Sporotrichiella, Gibbosum, Discolor subsection Saubinetii, Martiella, and Elegans subsection Oxysporum (excluding vascular parasite group).

10. Initiation of sporulation in *F. culmorum* was hastened. Irradiated plates showed as high as 83 per cent macrospores whereas controls showed no conidia, and even after the controls began to sporulate, treated plates showed a higher percentage of macrospores.

11. A strain of *F. coeruleum*, which had never produced conidia during its period of culture in this laboratory, and one of *F. sambucinum*, which was temporarily in "abkultur," were induced to produce macrospores after irradiation, although the controls were still sterile.

12. One strain of *Fusarium* (section Gibbosum), isolated from decayed onion bulbs, which had never produced spores in the laboratory, produced many spores (75 per cent macrospores) when irradiated. Subsequent irradiation failed further to increase this percentage.

13. There was little or no cumulative increase in macrospore percentage due to repeated irradiation of non-responsive species, although the quantity of spores present could be thus increased.

14. *F. vasinfectum* var. *zonatum* and *F. semitectum* gave maximum macrospore production under Corex filter 980 A transmitting to 2300 A.u. With *F. sporotrichioides* and *F. bulbigenum* the peak of macrospore production occurred under Corex 986 A transmitting between 2535 and 4340 A.u.

15. Most species tested gave maximum macrospore production under vitaglass. All responsive species gave good results under vitaglass.

16. A 15-minute irradiation period on each of three successive days was better than one longer period. A shorter period was usually not so effective. In some species increasing the length of period to one half-hour, or multiplying the number of 15-minute exposures was advantageous, in others injurious.

17. A distance of 40 cm. from the arc proved best for the purposes of these tests.

18. Exposure directly to the mercury arc, transmitting to 2230 A.u., resulted in decreased growth rate (as measured by radial increase), colony stunting, and injurious effects to mycelium and spores in all species. The extent of the injurious and lethal effects varied with the fungus. In some forms spore formation was inhibited, in others those produced were killed, or injured or deformed. Plasmolysis of protoplasm and shattering of spores occurred.

19. Most forms tried were injured slightly or not at all by the exposure to waves transmitted through the filters, though decrease in growth rate occurred with some species under some filters.

20. Increased macrospore production did not seem to be directly correlated with inhibition in growth rate.

21. A stimulation of macrospore production resulted upon irradiation of old mycelium in cultures of responsive species, although the mycelium already completely filled the petri dish and was checked in growth.

22. *F. argillaceum* usually produced many chlamydospores as a result of irradiation. Cultures exposed directly to the arc sometimes went over almost entirely to chlamydospore production.

23. In some species, as in *F. vasinfectum* var. *zonatum*, macrospore content was greatest 48 hours after irradiation ceased, and there was a drop in percentage subsequently owing to the production of micro-

spores on germination of the macrospores. In other species, as in *F. bulbigenum*, maximum macrospore percentage occurred 24 hours after the third exposure and a drop in percentage was noticeable the second day.

24. Neither three 15-minute exposures through vitaglass at 40 cm. from the arc nor three (or less) 4-second direct exposures at 21 cm. from the arc succeeded in inducing the production of perithecia of the 14 species with known perfect stages which were treated in these tests. Except for two plates in one test of *F. javanicum* var. *theobromae*, none of the cultures tested produced perithecia. In these two plates a few perithecia were present two months after irradiation in mycelium developed since irradiation.

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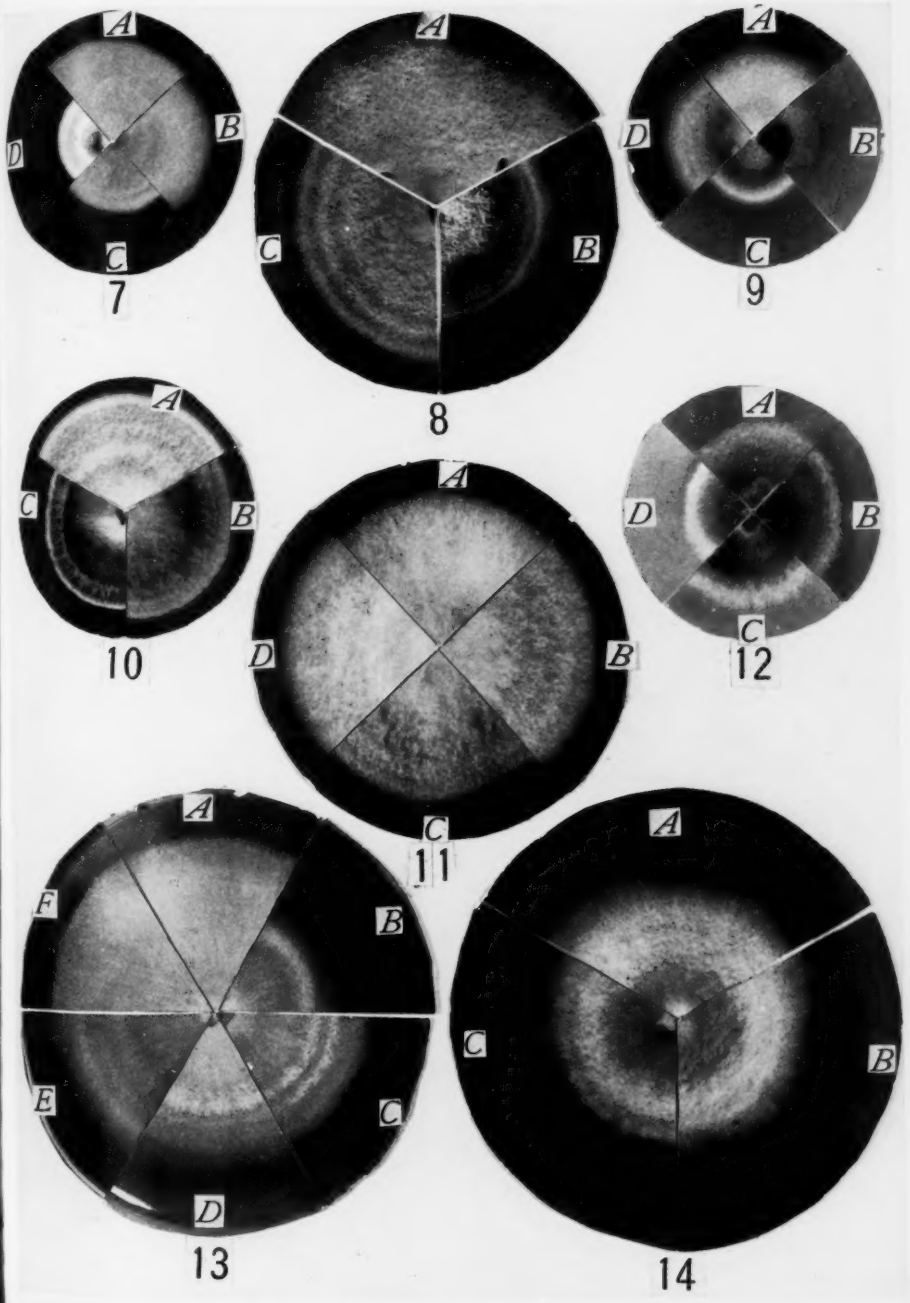

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EXPLANATION OF PLATE I

Segments of petri-dish cultures of *Fusarium* spp. showing comparative growth rates as affected by three daily 15-minute exposures to ultraviolet rays transmitted by quartz mercury arc through various filters (distance 40 cm.).

FIG. 7.—*F. javanicum* var. *theobromae:* A, control grown in diffuse light; B, exposed through vitaglass; C, exposed through Corex red-purple filter (986 A); D, exposed direct.

FIG. 8.—*F. bulbigenum:* A, control; B, exposed through filter 986 A; C, exposed through vitaglass.

FIG. 9.—*F. coeruleum:* A, control; B, exposed direct; C, exposed through Corning Corex filter (980 A); D, exposed through vitaglass.

FIG. 10.—*F. javanicum:* A, exposed through filter 986 A; B, exposed through filter 980 A; C, exposed direct.

FIG. 11.—*F. javanicum:* A, control; B, exposed through filter No. 1; C, exposed through vitaglass; D, exposed through Noviol C.

FIG. 12.—*F. neoceras:* A, control; B, exposed through vitaglass (no stunting); C, exposed through filter 986 A and showing no stunting; D, exposed through filter 986 A but showing stunting. Both plates sporulated equally well.

FIG. 13.—*F. vasinfectum* var. *zonatum:* A, control; B, exposed direct; C, exposed through filter 980 A; D, exposed through filter 986 A; E, exposed through vitaglass; F, exposed through filter No. 1.

FIG. 14.—*F. sporotrichioides:* A, control; B, exposed through vitaglass; C, exposed through filter 986 A.

GAMETOGENESIS IN VAUCHERIA

JOHN N. COUCH

(WITH THIRTY-FIVE FIGURES)

Introduction

A review of the literature on gametogenesis in *Vaucheria* indicates that there are two important problems connected with this process about which there is still considerable doubt. First, is fertilization effected in all species by means of motile sperms, or does the antheridium discharge directly into the oogonium? Second, what happens to the supernumerary nuclei in the oogonium? Do they migrate back into the thread as maintained by OLTMANNS (**11**) and HEIDINGER (**6**), or do they degenerate in the oogonium as claimed by DAVIS (**4**) and MUNDIE (**9**)?

VAUCHER (**16**) seems to have been the first who in any way understood the development and function of the sexual organs of *Vaucheria*. According to WALZ (**17**) the female sexual organ had been seen before VAUCHER, although it was not understood. VAUCHER discovered the male organ. Concerning fertilization he says (free translation):

> At first the antheridium is straight and opaque as a consequence of the contained fertilizing matter; little by little it curves toward the oogonium in order to deposit the fertilizing element on the latter. When the fertilizing element is discharged, the antheridium is empty and twisted in a spiral.

NÄGELI (**10**) was the next to attempt to describe the sexual process in *Vaucheria*. His observations are interesting chiefly from a historical standpoint. NÄGELI considered the antheridia and oogonia as organs of fertilization, the process being similar to that in the Zygnemaceae (fig. 1).

PRINGSHEIM (**12**) described accurately for the first time the development of the sexual organs. Studying the same species as NÄGELI (*V. sessilis*), PRINGSHEIM saw the formation of the sperms, their discharge, and fertilization. So accurate were his observations on living material that even today one can add little to his account

of the development of the sperms and fertilization. Quoting from a free translation:

> After the formation of the dividing wall in the antheridial branch ("Hornchen"), the colorless slime in its end gradually takes on a more distinct form, and one can easily recognize a great number of little rods in different places, close together and lying on top of one another, entirely colorless, which are still here and there surrounded by slime as though imbedded in it. The sharp observer will not miss the indistinct movement which some of these little rods show, and which gives a foreboding of their destiny.

PRINGSHEIM describes the discharge of the sperms:

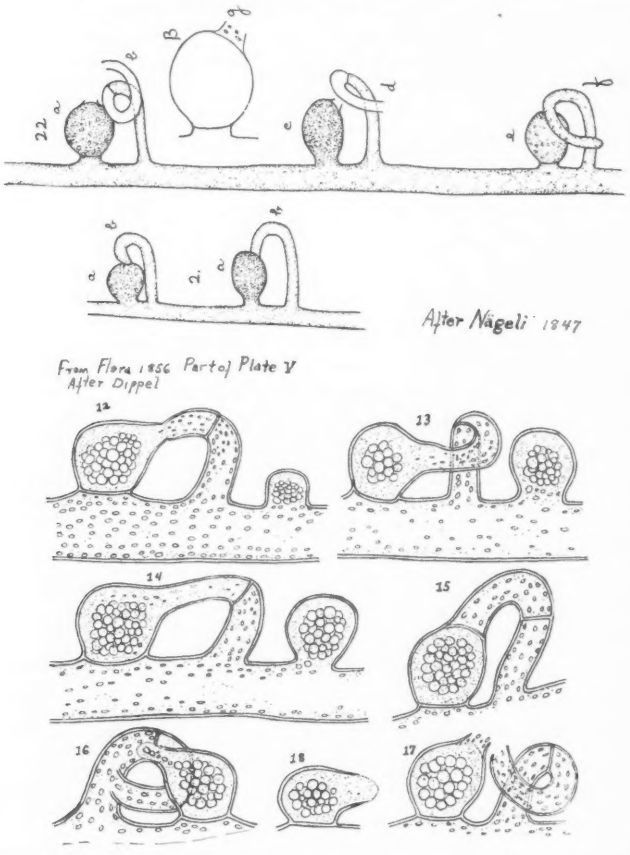
> Then always immediately after the opening of the oogonial beak, in wonderful coincidence, the antheridium opens at the end and pours out its contents. Countless extremely small, rod-shaped bodies emerge, some isolated and others still in the slime in which they were imbedded. Those already isolated glide about in all directions with incredibly quick movements; those imbedded in the slime gradually become free and follow with equal speed. Soon the field of view is covered with the minute, moving rods. In considerable numbers, 20, 30, and more, they push into the nearby opening of the spore fruit, which they completely fill.

He further describes how these sperms in the beak process of the oogonium push against the surface of the egg, attempting to penetrate it: "The sperms strike violently, fall back, push forward again, fall back again, and so continue in uninterrupted succession their pushing forward and falling back." He did not observe the actual penetration of a sperm into the egg, but saw what he took to be a sperm just after it had entered the egg.

The observations of PRINGSHEIM on *Vaucheria sessilis* are of interest because they are the first record of the union of a sperm with an egg in plants. The following year (1856) PRINGSHEIM actually watched the sperm enter the egg in *Oedogonium* (JOHNSON **7**).

DE BARY (**1**) described the development of the oogonia and antheridia and fertilization in *Vaucheria aversa*. He too observed the movement of the sperms in the antheridia, their discharge, and the probable entrance of a sperm into an egg. In the same year (1856) DIPPEL (**5**) maintained that PRINGSHEIM was wrong and NÄGELI right in the development of *Vaucheria*. He claimed that the so-called "Hornchen" (antheridium) of PRINGSHEIM fused with the beak of the sporangium (oogonium); that the cross walls between these

two organs disappeared; and that part of the contents of the "Hornchen" passed over into the sporangium (oogonium). After this the "Hornchen" broke away from the oogonial beak, bending around



FIGS. 1, 2.—Fig. 1 (above), NÄGELI'S figures of conjugation in *Vaucheria*. Fig. 2 (below), some of DIPPEL'S figures of conjugation in *V. sessilis*.

and assuming such a position that one could hardly suspect their former connection. The "Hornchen," however, did not always become separated from the oogonium but sometimes remained at-

tached. DIPPEL'S observations were made on *V. sessilis*, of which he presented a number of figures in support of his view. Figure 2 shows part of one of DIPPEL'S plates, illustrating fertilization in *Vaucheria*.

The observations of PRINGSHEIM and DE BARY were confirmed by SCHENK'S (**13**) study of *V. geminata*.

WALZ (**17**) described fertilization in *V. sericea* Lyngb. His work is in essential detail a confirmation of that of PRINGSHEIM, DE BARY, and SCHENK. He seems to have been the first actually to observe the penetration of the sperm into the egg in *Vaucheria*.

SOLMS-LAUBACH (**15**) described the development of the sexual organs and fertilization in *Vaucheria dichotoma*, and WORONIN (**18, 19**) did the same for *V. synandra* and *V. de baryana*, both writers corroborating, in a general way, the observations of PRINGSHEIM, DE BARY, and others. WORONIN (**18**) described the sperms of *V. synandra* as being somewhat elongated bodies, 5 to 6μ in length, with two cilia arising from near the anterior end, one being directed forward and the other backward. According to WORONIN, the sperms contain one or two very small, shiny, colorless bodies, corresponding perhaps to the red pigment dots which were seen by DE BARY and WALZ in *V. aversa*.

The observations so far described were made almost entirely on living material. The new killing, fixing, and staining technique developed about this time enabled SCHMITZ (**14**) to demonstrate the nuclei in *Vaucheria* for the first time.

The second problem in *Vaucheria* concerns the fate of the supernumerary nuclei in the oogonium. SCHMITZ stated that the young oogonium contained many nuclei and that the young oospore contained a single large nucleus. He did not offer any explanation, however, as to the fate of the extra nuclei. Later he claimed (according to MOTTIER **8**) that, in the plasmic mass given out upon the opening of the oogonium, small nuclear fragments were present which had probably become separated from the nuclei of the young oogonium.

BEHRENS (**2**) stated that the numerous nuclei in the oogonium fused to form one. He also noticed but did not understand the significance of the "Wanderplasm" which OLTMANNS later described.

OLTMANNS (**11**), using modern cytological technique, was the first to give a complete account of the development of the sexual organs,

including the nuclear behavior. He described in great detail the development of the sperms in *V. clavata* (*V. sessilis*), *V. fluitans*, and *V. aversa*, in both living and fixed material. According to OLTMANNS, the young antheridium contains several large vacuoles bordered by a rather dense cytoplasm in which many nuclei are imbedded. These nuclei elongate and become spindle-shaped, one end extending out into the vacuole and the other end being imbedded in the peripheral cytoplasm. Each of these nuclei with a small amount of surrounding cytoplasm becomes a sperm. Cilia may sometimes be observed on the sperms. Shortly before the antheridium opens, the sperms may be seen in motion within it. When the antheridium opens, the sperms emerge with great rapidity. The peripheral plasma layer may remain partly within the antheridium, even after the sperms are discharged. The antheridia generally open between 2 and 4 o'clock in the morning. The most remarkable of OLTMANNS' observations was in the development of the oogonia. The young oogonia contain a great number of nuclei, one of which is selected for the egg nucleus while the remaining nuclei migrate back into the vegetative threads. Fertilization occurs by the penetration of a sperm and the fusion of sperm and egg nucleus.

DAVIS (**4**), working with fixed material of *V. geminata*, studied the fate of the supernumerary nuclei in the oogonia. He claimed that the young oogonia were multinucleate, but that, contrary to OLTMANNS' observations, the supernumerary nuclei degenerated instead of migrating back into the vegetative thread.

HEIDINGER (**6**) studied the development of the sexual organs in *Vaucheria pachyderma*, *V. arrhyncha*, *V. terrestris*, and *V. geminata*. In *V. arrhyncha* he followed the development of the sperms and found that those from a single antheridium may take 15–20 minutes to emerge. In all four species he described the supernumerary nuclei as migrating back into the main thread. His work in general corroborates and extends that of OLTMANNS.

MUNDIE (**9**) was unable to find the sperms in *V. geminata*, although he killed material quite early in the morning (2:30 A.M.). He thought that perhaps fertilization might be effected by the antheridium and oogonium coming in close contact, a view which has been held also by NÄGELI (**10**), DIPPEL (**5**), and KARSTEN (see WALZ **17**).

MUNDIE'S observations in regard to the fate of the supernumerary nuclei seem, according to his interpretation, to corroborate those of DAVIS, in that he is of the opinion that they degenerate. It is my opinion that MUNDIE'S figures, which apparently were drawn with painstaking precision, may be given a different interpretation from that which he has stated. This matter will be discussed later.

Observations

VAUCHERIA SESSILIS

Excellent material of *V. sessilis* in fruit was collected from a small marsh in Chapel Hill, N.C., in January, 1930. This material was kept growing in petri dishes in the laboratory for several days, during which time it bore an abundance of sexual organs. Other lots of material in about the same condition were collected from the same locality several times during the winter and spring of 1930. The sexual organs cease development if the threads are injured to any extent, but by a simple manipulation it was possible to transfer threads to a slide for microscopic observation. Sexual organs in the desired stage of development were located under the low power of the microscope, a cover glass was dropped in the dish by the desired threads, and then under a binocular dissecting microscope with glass needles the threads were dragged carefully through the water on to the cover glass. The cover glass was then lifted out and placed with the material upright on a slide and observed thus without a cover. Material handled even so carefully aborts frequently, and it was found that very satisfactory observations can be made without removing the material from the dish if the threads bearing the sexual organs are gently teased out from the entangling mat.

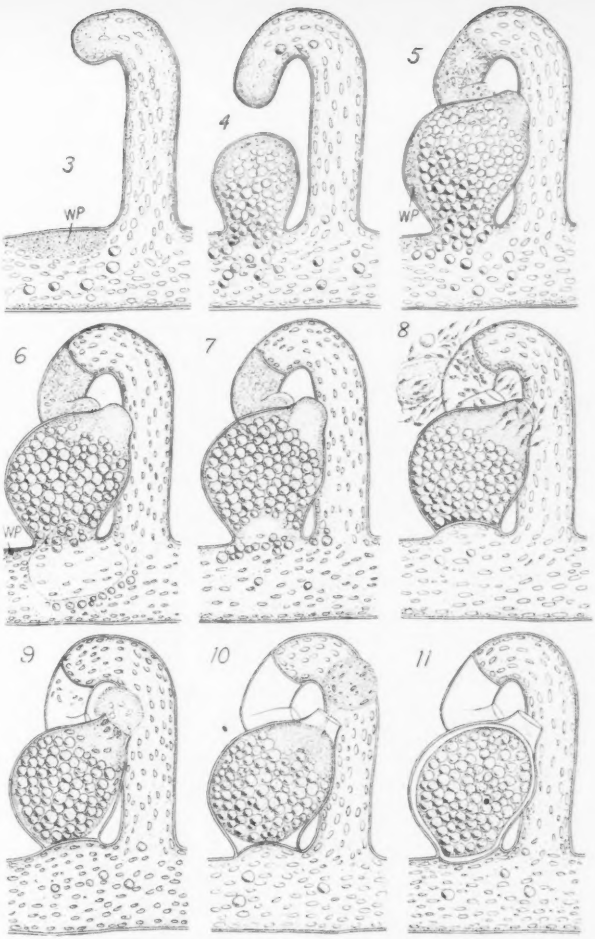
OLTMANNS found a very interesting rhythm in the development of the sexual organs in *V. sessilis*, and I have noticed the same phenomenon. This rhythm may be somewhat upset, however, after the material has been in the laboratory for several days. The antheridia start development first and become rather long and curved before the oogonia appear. The antheridia usually start their development in the afternoon, the oogonial branch sprouting in the early part of the night. The following afternoon the oogonial beak appears, the

sperms are discharged, and fertilization occurs sometime during the night, usually after midnight and before 3 A.M.

Since the observations of PRINGSHEIM and OLTMANNS on this species are so detailed, it is unnecessary to repeat their full account except where new or controversial points are concerned.

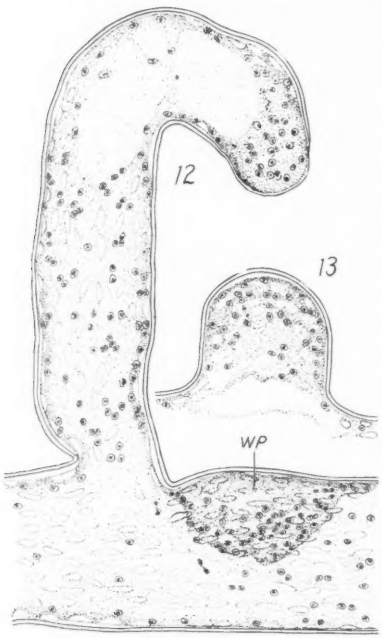
I have noticed a distinct lashing movement of delicate threads in the antheridial branch a considerable time before the antheridium is cut off. This movement occurs not only in the tip of the antheridial thread in the region which is to become the actual antheridium, but also for a considerable distance down the stalk. In one antheridial branch which was studied particularly on this point, this lashing movement was evident for about two hours before the antheridial wall was formed. These delicate threads extend from the thick cytoplasmic lining out into the vacuoles; rarely one may extend entirely across a vacuole. This movement suggests that the sperms are already forming in the dense cytoplasmic lining. At a later stage, after the cross wall separates the antheridium from the stalk, several (2 or 3) large vacuoles containing many sperms may be noticed, each with two cilia pointed in opposite directions, one of which is still anchored in the cytoplasmic membrane (fig. 5). Shortly before the antheridium opens, the sperms may be seen in vigorous motion, still inclosed in the vacuoles but no longer attached to the cytoplasmic membrane.

In the earliest stages in the development of the oogonia of *V. sessilis*, a very interesting condition has been noticed which has apparently escaped the attention of other observers. Before the main thread has even begun to bulge out to form the oogonial initial, a heaped-up mass of cytoplasm is found near the base of the antheridial stalk. This mass of cytoplasm may sometimes be so large as to extend over one-third of the way across the interior of the thread. It is more or less free from chloroplasts, and in the early stages does not seem to contain nuclei in the region next to the wall, but contains many nuclei in the region toward the center of the thread (figs. 3, 12, 13). This is OLTMANNS' "Wanderplasm" in its earliest stage of recognition. Later, as the thread bulges out, forming the oogonial initial, this mass of cytoplasm occupies the distal growing region, and



FIGS. 3–11.—Development of sexual organs of *V. sessilis* in living material from shortly after appearance of antheridial branch to mature zygote; observations from April 1 to 6, 1930: Fig. 3, young antheridium and "Wanderplasm" (*wp*), latter in position from which oogonium will sprout, April 1, 9:30 P.M. Fig. 4, young oogonium with peripheral "Wanderplasm," April 2, 8:30 A.M. Fig. 5, oogonial beak formed, "Wanderplasm" retreating into thread; oil globules passing into oogonium; antheridial wall forming, chloroplasts in antheridium degenerating; April 2, 9:30 P.M. Fig. 6, "Wanderplasm" entirely out of oogonium; large vacuole passing in; antheridial wall formed and sperms evident, some free in vacuoles, others imbedded in cytoplasm; April 3, 12:10–12:15 A.M. Fig. 7, basal wall of oogonium forming; sperm very distinct in antheridium; tip of antheridium undergoing hydrolysis; April 3, 12:30–12:40 A.M. Fig. 8, sperms emerging and swarming around oogonial beak. Some swam vigorously for about 14 minutes; together with the sperm, some cytoplasm and oil globules were discharged; April 3, 1:10 A.M. Fig. 9, oogonial membrane broken at tip and cytoplasm exuded; April 3, 1:50 A.M. Note sperms near tip of oogonium; these had crawled about over beak in an amoeboid-like fashion since about 1:25. Shortly after oogonium opened, the sperm disappeared. Fig. 10, cytoplasm extruded from oogonium, rounded up, and separating from egg; sperm apparently entering egg; April 3, 2 A.M. Fig. 11, ripe zygote completely filling old oogonial wall; April 6, 1930. All ×325.

stained preparations show that it contains many nuclei (fig. 13). After the oogonial beak is formed, this hyaline area of protoplasm moves to the side of the oogonium opposite the beak, and slowly retreats into the thread (figs. 4, 5).



FIGS. 12, 13.—*V. sessilis* killed and stained (Gram's method) *in toto*, showing young antheridium and "Wanderplasm." Fig. 12 (combined surface and section view), note "Wanderplasm" by base of antheridium: its outer region is composed of cytoplasm while toward center of thread are vast numbers of nuclei. Fig. 13, oogonial branch sprouting, mostly filled with "Wanderplasm." Section view ×625.

After the "Wanderplasm" has entirely emerged from the oogonium, a conspicuous vacuole appears in the thread at the base of the oogonium (fig. 6). This vacuole may remain in this position for 10–15 minutes or even longer, or it may pass immediately into the oogonium. A few minutes after the vacuole passes in, vague signs of a basal membrane become visible, and in a few minutes more, as a rule, the basal wall is formed. In the oogonium shown in the accompanying figures, no record was made of the time when the "Wanderplasm" had entirely emerged; the vacuole, however, passed in between 12:10 and 12:15, and by 12:40 the basal wall had been formed. The same sequence of events was observed several times in *V. pachyderma* and *V. aversa*. OLTMANNS does not mention the vacuole, and according to him about an hour elapses between the moment when the "Wanderplasm" has completely emerged and when the basal wall is completed.

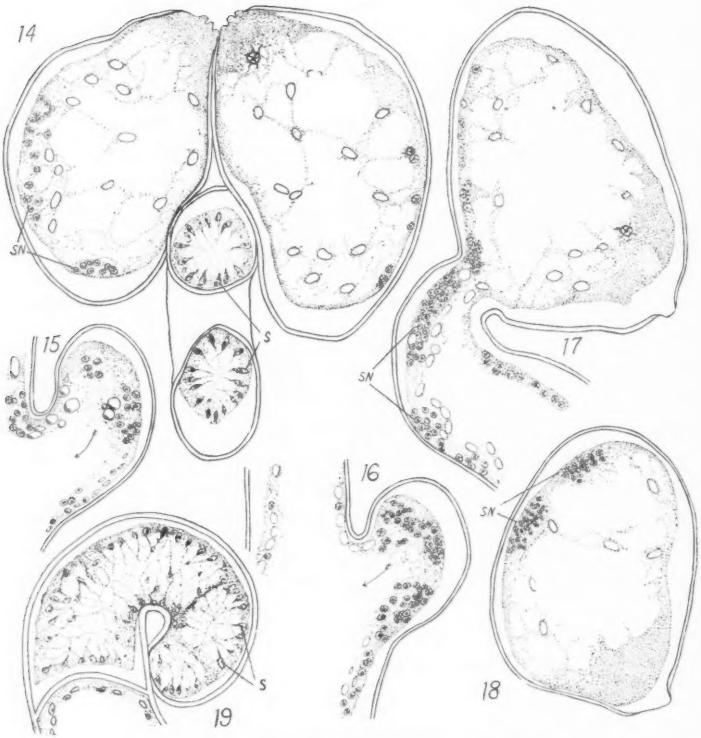
I was unable to get stained sections of the oogonia at this stage to determine whether this retreating protoplasm contained the super-

numerary nuclei, as maintained by OLTMANNS. So far as they go, however, these observations clearly substantiate his.

PRINGSHEIM stated that the oogonium and antheridium opened almost simultaneously, the oogonium opening slightly before the antheridium. I have observed the opening of a great number of oogonia and antheridia, and while adjacent ones may open almost simultaneously, it happens just as frequently that either one or the other may open from a few minutes to two hours before the other opens, a condition which would tend to prevent self-fertilization. HEIDINGER describes a sort of intermittent discharge of the sperms in *V. arrhyncha*. This period of discharge from one antheridium lasts from one-quarter to one-half hour. In *V. sessilis* a somewhat similar condition obtains, although the period of discharge does not last so long as in *V. arrhyncha*. It often happens, when the antheridium opens before the oogonium, that some of the sperms, after swimming around the oogonial beak for several minutes, may come to rest on the beak, crawling about over its surface in a somewhat amoeboid fashion. The actual penetration of the sperm into the oogonium has not been observed with absolute certainty in living material, but several stages in this act have been found in fixed preparations.

VAUCHERIA GEMINATA

This species was collected from the same location as *V. sessilis*. In spite of the fact that a considerable number of continuous observations were made on the development of the sexual organs in this species, the discharge of the sperms was never observed. At one time the same antheridium was kept under observation from early afternoon until 5 A.M. the following morning, at which time it was still unopened. In such antheridia the movement of cilia-like structures was observed, and great numbers of completely empty antheridia were seen. In material killed about 3 A.M. and stained *in toto*, or sectioned and stained (iron-alum haematoxylin or Gram's gentian violet method), the sperms could be clearly seen (figs. 14, 19*s*). In the sectioned material the sperms were arranged exactly as described by OLTMANNS for *V. sessilis;* that is, one of the ends of the sperms was fastened in the cytoplasmic layer, and the free ends, each with a single cilium attached, extended out into the vacuole (fig. 14). In



FIGS. 14–19.—*V. geminata:* Fig. 14, horizontal section through two oogonia and one antheridium, showing enlarged female gamete nucleus in oogonium to right; female gamete nucleus also present in oogonium on left but not in section drawn. Note supernumerary nuclei (*sn*) and degenerating chloroplasts, which might be confused with nuclei in poorly stained preparations, scattered throughout the cytoplasm. Two sections of antheridium shown, both with distinct sperms with one end fastened in cytoplasmic membrane. Fig. 15, showing stalk of oogonium on right, and clusters of nuclei which have passed out of oogonium. Fig. 16, stalk of same oogonium nearer thread, and showing vast numbers of nuclei migrated from oogonium. Fig. 17, section through oogonium and stalk showing female gamete nucleus. (Many oogonia were seen in this condition with the female gamete nucleus invariably in this position.) Note large number of nuclei which have migrated from oogonium. Fig. 18, section of same oogonium as shown in fig. 17, showing clusters of nuclei; very few nuclei showed signs of degenerating. Fig. 19, antheridium stained *in toto* with iron-alum haematoxylin; sperm not so far developed as in fig. 14. All ×625. All material killed 3 A.M.

the sketch of the entire antheridium (fig. 19) the sperms are not so advanced in their development as shown in figure 14. My failure, as well as MUNDIE'S, to observe the discharge of the sperms in *V. geminata* was doubtless due to insufficient observations.

Because of the position of the oogonia, it is very difficult to follow the "Wanderplasm" in this species in living material. Several cases were seen, however, which showed a very distinct "Wanderplasm" apparently passing from the oogonium back down the thread.

In fixed material and preparations stained *in toto*[1] a number of oogonia were found which showed clearly that most of the nuclei pass back into the threads, but no single oogonium was found which did not still contain a considerable number of nuclei. In such oogonia the supernumerary nuclei are disposed near the wall, almost invariably being arranged in clusters (figs. 14, 18). Although great numbers of oogonia have been examined at this stage, the nuclei have never been found scattered throughout the cytoplasm as figured by DAVIS. In several slides there were sections of *V. terrestris* showing well stained nuclei scattered through the cytoplasm somewhat as shown in DAVIS' figures. DAVIS shows only 9–15 nuclei in his sections of oogonia, whereas in sections which show the "Wanderplasm" well the number of nuclei is much larger. In some oogonia many of these supernumerary nuclei are peripherally disposed, and are arranged in such a way as to be apparently going to take part in the formation of the egg wall. Such nuclei often show various stages of degeneration. Unfortunately, since all of the material was killed at 3 A.M., all of the oogonia which are developing are in almost exactly the same stage. All show a single, enlarged egg nucleus in the

[1] The method used in preparing fixed material of both *V. geminata* and *V. aversa* for study was as follows: Kill in Chamberlain's chrom-acetic solution for *Vaucheria* 6 hours; wash 24–48 hours in still water; dehydrate with 5–100% alcohols; transfer to xylol $\frac{1}{4}$-alcohol $\frac{3}{4}$ 30 minutes, xylol $\frac{1}{2}$-alcohol $\frac{1}{2}$ 30 minutes, xylol $\frac{3}{4}$-alcohol $\frac{1}{4}$ 30 minutes, xylol 30 minutes; then transfer to xylol and paraffin, then paraffin 2 hours; imbed in 58° paraffin. Section 6–10 μ thick; clear; descend in alcohols to water; stain in aniline gentian violet 10 minutes; dip several times in water to remove surplus stain; treat with iodine-potassium iodide solution 2–5 minutes; dip several times in 95% alcohol (not over 30 seconds), 100% alcohol 1–2 minutes, clove oil (flood slide 2 or 4 times), cedar oil, xylol 5 minutes; and mount in balsam.

For more complete details on GRAM'S method, see COUCH, J. N., The development of the sexual organs in *Leptolegnia caudata*. Amer. Jour. Bot. **19**:584–599. 1932.

dense region of cytoplasm at the beak. The egg nucleus, however, is invariably in the margin of the cytoplasm toward the center of the oogonium (figs. 14, 17). MUNDIE (figs. 2, 4, 11, 14) shows the female gamete nucleus in about this position. One of his figures, however, shows the nucleus rather close to the tip (MUNDIE, fig. 13). DAVIS describes and figures the female gamete nucleus in the center of the egg. Since the basal wall had not been formed in any of these oogonia, it is not possible to say what is the ultimate fate of the supernumerary nuclei which are still in the oogonium at this time.

MUNDIE killed his material at 2:30 A.M., while mine was killed at 3 A.M. It is interesting that the oogonia in his material and in mine should have been so nearly in the same stage of development. MUNDIE finds the supernumerary nuclei in the oogonia in much the same position as here described, that is, they are in the position where the "Wanderplasm" is supposed to be. He also shows a few nuclei back in the stalk which may have migrated from the oogonia. These nuclei in the stalk are so abundant and conspicuous that, if the material is killed at the right time and properly fixed and stained, one cannot fail to observe them (figs. 15–17). It is probable that most of the supernumerary nuclei figured by MUNDIE would have passed out of the oogonia if his material had been killed an hour or so later.

There seems to be considerable confusion as to the time when the cross wall is formed. DAVIS, working entirely with fixed material, states that the wall appears when the oogonium is about two-thirds its mature size, while MUNDIE says that the cross wall is not formed until after fertilization. Unfortunately I cannot say precisely when the cross wall is formed in this species. Examination of a great number of longitudinal sections through the oogonium and stalk, as in figure 17, indicates that the basal wall is not formed until a considerable time (several hours) after the oogonium attains its mature size. Of course, if the basal wall were formed when the oogonium is only two-thirds mature in size, as claimed by DAVIS, the supernumerary nuclei would be held in the oogonium and could not retreat into the thread. The migration of the "Wanderplasm" does not begin in *V. sessilis*, *V. pachyderma*, and *V. aversa* until the oogonia have reached mature size, and it is probable that the same sequence of events occurs in *V. geminata*. MUNDIE also is almost certainly wrong as to

the time when the basal wall is formed. His figures 11 and 14, intended to show stages in fertilization, are almost certainly some considerable time before that act. The oogonia have not opened at the tip, an occurrence which invariably precedes the entrance of the sperms in all other species which have been investigated; nor does either of the oogonia (MUNDIE'S figures 11 and 14) show any sort of perforation in the wall through which the sperm substance might have been transferred. It is very probable that the basal wall in *V. geminata* is formed in much the same way and at about the same time (about an hour or so before the oogonium opens) as in *V. sessilis*, *V. pachyderma*, and *V. aversa*.

VAUCHERIA PACHYDERMA

In *V. pachyderma* there is considerable "Wanderplasm," and hence this species is one of the best for observations on the fate of that substance. On March 25, 1930, and for several days thereafter, excellent fruiting material of *V. pachyderma* was found growing on a damp bank in the arboretum at Chapel Hill, N.C. Several continuous series of observations on the development of the oogonium and antheridium were made, all with essentially the same results. On the first series observations were begun at 4 P.M., and except for about 45 minutes' absence were continuous until 4 A.M. Twelve camera-lucida sketches were made at intervals of about an hour to show the changes through which the oogonium and antheridium passed. Four of these sketches are reproduced in figures 20–23.

The oogonium and antheridium, selected for observation (fig. 20), were mature in size at 4 P.M., when the observations were begun. Both organs were still in communication with the main thread, although signs of a membrane separating the more or less colorless contents of the antheridium from its stalk had become apparent. The antheridium contained granular cytoplasm in the center of which was a large vacuole. Close to the membrane delimiting the antheridial cytoplasm from that of the stalk were numerous disintegrating chloroplasts. There were also a few scattered here and there through the antheridial cytoplasm. The antheridium contained little or no oil.

The oogonium had developed a distinct beak which contained

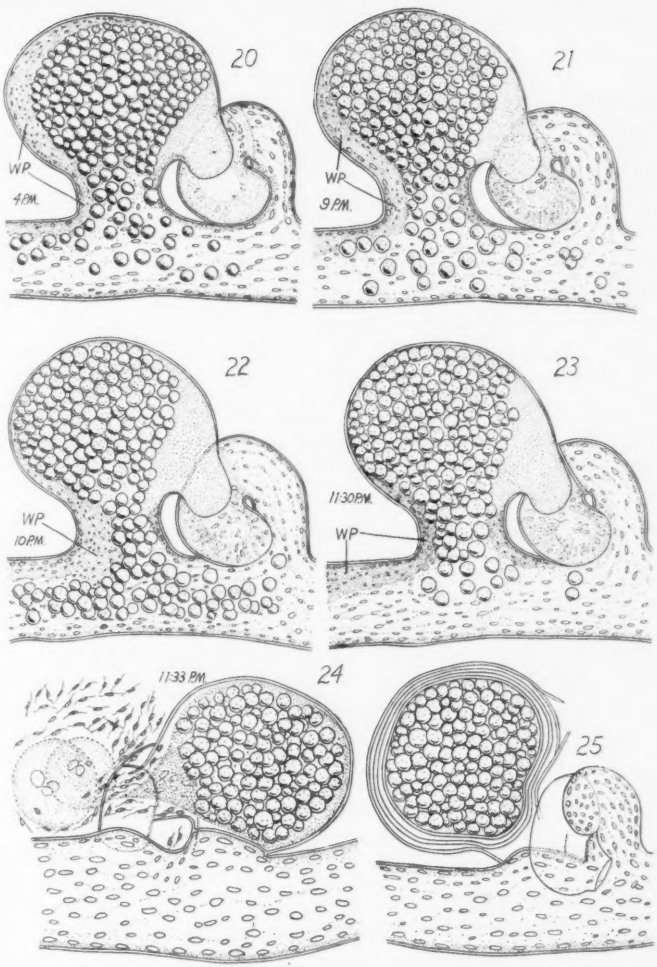
colorless cytoplasm. Two or three rather distinct granules, which were sometimes apparently furnished with astral rays, could be seen in the oogonial beak. The central part of the oogonium was apparently occupied by a large vacuole. On the sides of the oogonium, oil globules were collected in great numbers. At the back, opposite the beak and extending from the top down to the neck, was a dense layer of granular cytoplasm containing a very few scattered chloroplasts (fig. 20). This dense layer of cytoplasm is the "Wanderplasm" of OLTMANNS and HEIDINGER.

At 8 P.M. the membrane separating the antheridium from the antheridial stalk had moved up considerably. A small vacuole had appeared near the tip of the antheridial cell, and a faint motion of cytoplasmic threads was visible in the vacuole. No perceptible change had taken place in the oogonium, except that the "Wanderplasm" had become thicker toward the base and in the neck. Attention was now concentrated on the "Wanderplasm," and immediately it was seen to be moving slowly outward into the stalk. This movement was exceedingly slow and was alternated with periods of apparent complete quiescence. The movement was best detected by focusing attention on one granule as it passed through the neck. By 9 P.M. the "Wanderplasm" had receded entirely from the top of the oogonium and was continuing to move out into the thread (fig. 21).

At this time three or four elongated rods could be seen moving autonomously about in the vacuole in the antheridial cell. These were evidently precocious sperms. Rodlike bodies were also becoming apparent here and there in the cytoplasm of the antheridium.

By 9:30 P.M. the "Wanderplasm" had moved out of the oogonium in such quantity that it occupied more than one-third of the space in the neck. This mass was divided into two rather distinct zones: an outer zone of pale green, finely granular cytoplasm and an inner zone of still paler green, hyaline cytoplasm with large granules. In the antheridium the vacuole had become somewhat more distinct, and now contained several elongated motile bodies.

At about 10 P.M. approximately half the space in the neck was occupied by the "Wanderplasm" (fig. 22). The movement continued, until by 12:30 A.M. the entire mass has passed from the oogonium back into the thread. At about 11 P.M., when the "Wander-



FIGS. 20–25.—*V. pachyderma:* Fig. 20, conspicuous "Wanderplasm" on top, back, and extending down into thread. Oogonium contains much oil; beak filled with clear cytoplasm; antheridial wall forming. Antheridium contains granular cytoplasm and degenerating chloroplasts; 4 P.M. March 26, 1930. Fig. 21, "Wanderplasm" receding from oogonium; sperm evident in antheridium; 9 P.M. March 26. Fig. 22, "Wanderplasm" mostly receded from oogonia; 10 P.M. March 26. Fig. 23, "Wanderplasm" practically entirely out of oogonium and oil globules passing into oogonium; 11:30 P.M. March 26. Fig. 24, antheridium and oogonium which opened almost simultaneously; 11:30 P.M. Fig. 25, oogonium with ripe zygote. All ×325.

plasm" had almost entirely receded from the oogonium, the oil globules in the thread were observed to be moving into the oogonium, the passage inward continuing until most of the oil which had accumulated in this region of the thread had moved into the oogonium (fig. 23). Between 11:30 and 12 the membrane between the antheridium and its stalk was replaced by a distinct wall. At this time close observation would reveal the sperms imbedded in the cytoplasm. The antheridium also still contained a few pale green chloroplasts. By 1:15 A.M. numerous sperms could be seen, both in the cytoplasm and in the vacuoles of the antheridium. About 1:15 A.M. a large vacuole passed from the thread into the oogonium, and almost immediately a membrane between the oogonium and its stalk became evident. This same sequence of events was observed in several other oogonia. No apparent noteworthy change took place in the oogonium and antheridium between 1:15 and 3 A.M., except that the vacuole changed its shape somewhat and the basal membrane of the oogonium became more distinct.

At 3:10 A.M. some fresh water was added to the *Vaucheria,* and in a few minutes the antheridium opened. Instead of discharging motile sperms, the contents of the antheridium slowly oozed out into a mass which assumed a spherical shape resembling the undifferentiated, discharged content of a *Pythium* sporangium. This mass of material consisted of undifferentiated sperms, cytoplasm, and a number of differentiated sperms some few of which succeeded in freeing themselves from the mass. The bladder containing the sperms remained at the tip of the antheridium until after 3:30 A.M., about which time it became separated from the tip and sank to the bottom of the dish. The oogonium had not opened at 4 A.M., at which time observations were discontinued. The following morning the oogonium still had not opened, but contained a mass of large oil globules.

In several other series of observations the normal opening of the antheridium and oogonium was observed. Suddenly the tip of the oogonial beak gives way and the hyaline cytoplasm begins to ooze out. The cytoplasm continues to stream out for one or two minutes, forming at first an elongated twisted mass. While this is coming out the antheridium usually bursts. In a few seconds more the mass of cytoplasm extruded from the egg assumes a rounded shape, becom-

ing separated from the long tapering strand of cytoplasm which extends out from the egg. Meanwhile the sperms are swarming about the mass of cytoplasm which has been extruded from the egg, and great numbers of them swim about the oogonial tip, some entering the space between the egg and oogonial wall (fig. 24). As soon as the extruded sphere of cytoplasm becomes separated from the egg cytoplasm, the protruding tip is drawn back into the egg. A membrane now forms around the egg and the latter swells considerably, completely filling the oogonium. Sometimes the egg may continue to swell so that another mass of cytoplasm is extruded, after which the tapering tip is drawn back into the egg, the tip rounding off as before. This has been observed three times in succession in the same oogonium.

I have been unable to observe a sperm actually enter the egg. Apparently this would most likely occur while the egg is in the condition shown in figure 24. The sperms remain active for only a short time (about 10 minutes) in this species. After a few days a thick wall is formed around the zygote and it becomes separated from the main thread (fig. 25).

Vaucheria aversa

Living material.—This species affords even more favorable material for studying the development of the sexual organs than *V. pachyderma*, particularly in regard to the development of the antheridium, since in *V. aversa* this organ is straight and is so placed that it is seldom obscured by the oogonium. This species also has a large quantity of "Wanderplasm," a characteristic facilitating observations on the fate of that substance.

Vaucheria aversa is rather common, although not abundant, around Chapel Hill, N.C., occurring from January to April in still or running water or on damp soil.

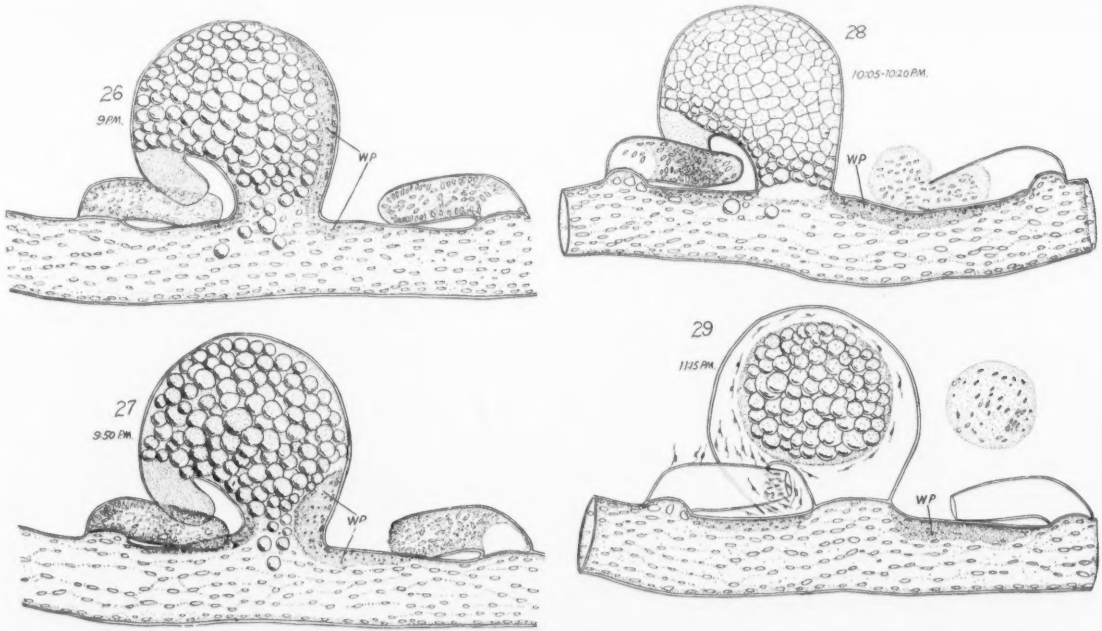
In this species the early development of the sexual organs was not studied, but observations were begun in the late afternoon or early evening on oogonia and antheridia which had reached mature size. Such an oogonium with its accompanying antheridia is shown in figure 26. The oogonium is comparatively very large, with a distinct beak which curves downward and inward toward the base of the oogonium. Each oogonium almost invariably has two antheridia,

both arising from the main thread, one in front of the oogonium and the other behind, and both extending toward the oogonium. The oogonium at this time (9 P.M.) contained many large oil globules which were mostly on the sides against the wall. The beak contained colorless cytoplasm, and toward the back there was a layer of more or less colorless cytoplasm which extended from the top down into the thread for some distance. This is the "Wanderplasm" of OLTMANNS and HEIDINGER. The central part of the oogonium was apparently occupied by a vacuole. At this stage of development both antheridia contained distinct sperms, most of which were imbedded in the peripheral cytoplasm.

By 9:50 P.M. the vacuole in the oogonium had increased in size and the "Wanderplasm" had mostly emerged from the oogonium. By focusing attention on the latter its slow movement out of the oogonium could be seen (fig. 27). Fifteen minutes later the "Wanderplasm" had completely emerged from the oogonium and the basal wall was beginning to be formed. The "Wanderplasm" was still evident out in the thread (fig. 28). The colorless cytoplasm in the beak region had increased in area. At this stage it appeared that the internal pressure in the oogonium was considerable, for the oil globules which heretofore had been more or less rounded now pressed against one another so that they appeared polygonal in shape. The beak also showed the effect of this pressure, straightening out somewhat.

At 10:05 one of the antheridia opened and the contents began slowly pouring out and forming a spherical mass at the tip of the antheridium. The contents passed out so slowly that it took 45 minutes for the antheridium to become completely empty. During this time a faint movement of the sperms was evident. At 11:12 the mass separated from the antheridium and sank to the bottom of the dish. At this time the sperms had become rather active in the mass. Several antheridia were seen to discharge in the same way as in *V. pachyderma* and *V. sessilis*.

About 11:15 the antheridium near the oogonial beak opened and the entire contents poured out in a few seconds (fig. 29). Simultaneously the oogonial beak burst and the contents shrank from the wall, rounding up in the center to form the egg. The sperms entered in



FIGS. 26–29.—*V. aversa*, showing fate of "Wanderplasm" and development and discharge of sperms. Fig. 26, "Wanderplasm" receding from oogonium; sperms evident in antheridium. Note large vacuole at base of antheridium at right; 9 P.M. April 6, 1930. Fig. 27, same oogonium 50 minutes later; very conspicuous "Wanderplasm" almost out of oogonium which contained large vacuole not shown in figure; sperm becoming more distinct. Fig. 28, same oogonium 15–30 minutes later; "Wanderplasm" entirely out but still visible in thread. Pressure in oogonium causing flattening of oil globules; basal wall of oogonium forming. Contents of antheridium on right passing out as in sporangia of *Pythium;* sperms distinct. Fig. 29, same oogonium 45 minutes later. All ×325.

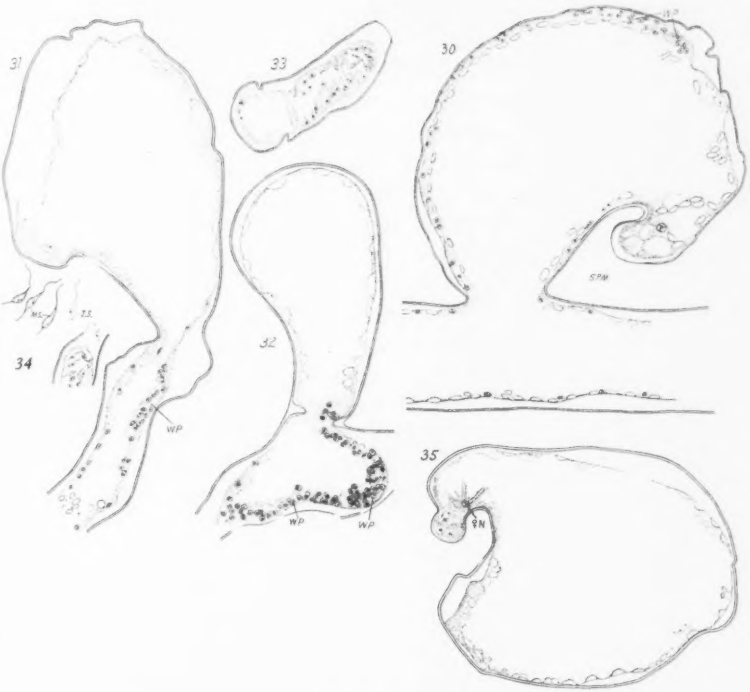
great numbers, swarming about the egg. I was unable, however, to see a sperm enter the egg.

FIXED MATERIAL.—Oogonial material of *V. aversa* was killed in Chamberlain's solution for *Vaucheria* at 5 and 11:30 P.M., April 6, 1930. Sections were cut 6 and 8μ thick and stained with an anilin solution of gentian violet and a solution of orange G in clove oil.

Sections of oogonia killed at 5 P.M. showed two very instructive conditions in regard to the fate of the "Wanderplasm." Several sections of oogonia were seen just before the oogonial beak was well formed and before the egg nucleus had become evident. In such oogonia there was a large central vacuole surrounded by a rather thick cytoplasmic layer. Numerous nuclei were scattered more or less evenly throughout the cytoplasm. Sections of other oogonia killed at the same time showed considerably later stages of development. Such a section is shown in figure 30. The oogonium had attained its mature size and the beak process had been formed. The beak was almost full of rather dense cytoplasm, in the center of which was a comparatively large nucleus, the female gamete nucleus. The central vacuole had increased greatly in size with a simultaneous thinning of the peripheral cytoplasm. The most remarkable change between this oogonium and the one just described, however, was in the position of the nuclei, which were located near the beak region. Immediately after the selection of the egg nucleus, the supernumerary nuclei began to recede from the region of the beak. This is strikingly shown in figure 30. In this oogonium, all the sections of which were well fixed on the slide, all the supernumerary nuclei had migrated from the beak region, passing backward toward the top of the oogonium. In this region there were many nuclei.

The supernumerary nuclei continued slowly to migrate backward from the oogonium until none but the female gamete nucleus was left. It appears as though the supernumerary nuclei are actually repelled by the female gamete nucleus. Material killed at 11:30 P.M. showed oogonia from which all the "Wanderplasm" with its contained nuclei had passed (figs. 31, 32). The number of nuclei which pass out is enormous. A count was made of the number which emerged from one oogonium and the number which appeared in four sections was 250. Two of the sections near the center were

lost. Allowing 75 nuclei for each of these sections, the total number would be about 400. This large number was perhaps due to the fact that the nuclei underwent almost simultaneous mitosis as they



FIGS. 30–35.—Fig. 30, section of oogonium of *V. aversa* showing egg nucleus and tip region of oogonium from which supernumerary nuclei have receded, accumulating toward top of oogonium (*wp*); killed 5 P.M. ×625. Figs. 31 and 32, sections of same oogonium from which nearly all the supernumerary nuclei (*wp*) have passed. Note egg nucleus in tip and scant amount of cytoplasm left in oogonium. Figs. 33 and 34, sections of the two antheridia accompanying this oogonium showing very distinct sperms; killed 11:30 P.M. All figures except free sperm in fig. 34, ×625; free sperm, ×1000. *ms*, mature sperm; *is*, immature sperm. Nucleus in immature sperm more distinct than in mature sperm. Fig. 35, oogonium with basal wall formed, supernumerary nuclei having all passed out; female gamete nucleus and few chromatin granules in tip, perhaps the remains of degenerated nuclei. ×625.

emerged. When the "Wanderplasm" has all emerged from the oogonium, a very scant amount of cytoplasm is left (fig. 35). My material, unfortunately, did not show stages in fertilization.

The emergence of the supernumerary nuclei, together with some of the cytoplasm, or "Wanderplasm," from the oogonia in *Vaucheria* is indeed one of the most remarkable phenomena yet described in the oogenesis of any of the Thallophytes. In fact it is so unusual that neither of the Americans (DAVIS, MUNDIE) who have worked on the cytology of *Vaucheria* have been willing to accept the evidence offered by OLTMANNS and HEIDINGER. It is easy to observe the fate of the "Wanderplasm" in *V. sessilis*, *V. pachyderma*, or *V. aversa* simply by following through the development of the oogonia in living material. Indeed since the development usually follows a definite rhythm, it is essential first to study living material in order to discover this rhythm, so that one may fix material in the desired stages of development. OLTMANNS, who first noticed this rhythm in the development of the oogonia, described it in great detail in his classic paper. Neither DAVIS nor MUNDIE appears to have made any continuous observations on living material, or to have known of the rhythmic development described by OLTMANNS, and this probably explains why they failed to observe the "Wanderplasm" in *V. geminata*.

So far as I have been able to discover, the only other plant which shows this peculiar economy in preserving the supernumerary nuclei, or part of them, during gametogenesis is *Endogone lactiflua*. In this fungus, as described by BUCHOLTZ (3), the large gametangium is multinucleate, containing one large gamete nucleus and many smaller ones. Before the basal wall is formed all or the greater number of the small supernumerary nuclei migrate back into the thread. The supernumerary nuclei which do not withdraw into the thread degenerate.

Summary

1. A survey of the available literature on sexual reproduction in *Vaucheria* from VAUCHER down to the present time is given. From this survey it appears that the sperms have been observed in all species investigated, with the possible exception of *V. geminata* (there is some doubt about the plant studied by SCHENK being *V. geminata*).

2. The development of the sperms and their discharge were ob-

served in *V. sessilis*, *V. pachyderma*, and *V. aversa*. In *V. geminata* the development of the sperms and their movement in the antheridium were observed, but the discharge of the sperms was not seen. In the species in which discharge was observed this usually occurred at night, sometime after midnight. The present observations on the development of the sperms corroborate those of PRINGSHEIM, WALZ, WORONIN, OLTMANNS, and HEIDINGER.

3. In *V. sessilis* the "Wanderplasm" initiates the formation of the oogonial branch, but as soon as the oogonium is about mature the "Wanderplasm" migrates back into the main branch.

4. In *V. geminata* the "Wanderplasm" passes back out of the oogonium, carrying most of the supernumerary nuclei. Although observations are incomplete, it appears that some of the nuclei may remain in the oogonium to degenerate.

5. In living material of *V. pachyderma* there is a large and conspicuous "Wanderplasm" which passes entirely back into the main thread.

6. Studies on living material of *V. aversa* gave essentially the same results as in *V. pachyderma*. In *V. aversa* stained material showed with apparent certainty that practically all of the supernumerary nuclei migrate back into the main thread with the "Wanderplasm."

7. In *V. sessilis*, *V. pachyderma*, and *V. aversa* the basal wall of the oogonium is formed from 15 minutes to an hour after the "Wanderplasm" has passed out, and from a few minutes to an hour before the oogonium opens.

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FURTHER STUDIES ON COLD RESISTANCE IN EVERGREENS, WITH SPECIAL REFERENCE TO THE POSSIBLE RÔLE OF BOUND WATER[1]

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Introduction

With few exceptions, the exposed portions of all plants living in habitats where temperatures fall below freezing at certain periods of the year must possess the property of cold resistance,[2] at least during the actual duration of such periods of subzero temperatures. The only portion of such plants which is normally not exposed to the prevailing atmospheric temperatures is the root system, and usually only a part of it escapes freezing temperatures. In some species the interior of massive structures such as tree trunks may also escape exposure to temperatures as low as those which may be reached in other parts of the same plant, owing to temperature lags. In many latitudes certain species of plants and parts of other species are blanketed under heavy snowfalls for most or all of the cold period of the year. Such plants or plant parts probably often do not develop the degree of cold resistance possessed by those exposed directly to low temperatures. Submerged water plants are another group in which the property of cold resistance, as the term is used in this paper, probably often does not develop.

In species which retain their leaves during the colder months of the year, the leaves as well as the exposed woody parts of the plant must possess this property of cold resistance. Such evergreen leaves are not, in general, resistant to cold during the warmer months of the year. The writer's experiments conducted with leaf-bearing branches of the pitch pine (*Pinus rigida* Mill.) have shown invariably that if they were exposed for periods of several hours to a tempera-

[1] Papers from the Department of Botany, the Ohio State University, no. 301.

[2] The term cold resistance as used in this paper refers only to the ability of plant tissues to survive unharmed periods of freezing temperatures which ordinarily would be expected to kill or severely injure them, owing to the formation of ice crystals. The term hardiness as usually applied to plants has essentially the same connotation.

ture of −20° C. during the winter months no damage resulted. Similar branches collected during the summer months and similarly exposed invariably showed a complete or nearly complete killing of the leaves on those branches. Similar results with other evergreens are described in detail by WINKLER (**32**). Evidently the property of surviving unharmed periods of freezing weather is possessed by evergreen leaves in general only during those periods of the year when such temperatures frequently prevail.

Apparently the leaves of evergreens such as the pitch pine go through an initial "hardening" process during the autumn months, and a corresponding final "dehardening" process during the spring months. In the latitude of Columbus, as an earlier investigation by the writer (**17**) has shown, this hardening process usually takes place during November, while the corresponding dehardening process usually occurs in May.

The preceding statements should not be understood to preclude the possibility that dehardening and hardening processes may also occur during the winter months. WINKLER, for example, found that the leaves of a number of evergreen species, all of which on January 2 withstood a temperature of −18° C. without injury, were practically all killed if branches of these evergreens were kept in a room at 20° C. for 16 days and then exposed to a temperature of −12° C. According to the work of TUMANOV (**30**), wheat plants lose a relatively large part of their cold resistance very quickly upon being brought into a warm room. For example, wheat plants hardened at 7° C. for 5 to 7 days lost nearly half of their relative cold resistance upon being kept in a room at 15°–17° C. for one day. The results of both of these investigations show that during warm periods in the winter a partial or complete loss of the ability to withstand temperatures below freezing occurs very commonly, at least in some species. With the onset of cooler weather, the leaves of such species again become hardened.

In another experiment TUMANOV showed that as short a period of exposure as one day to a temperature of 5° C. markedly increased the cold resistance of wheat plants, although maximum resistance developed only with longer periods of exposure (two to three weeks). In this species the plants pass out of the condition of hardiness much more rapidly than they gain it. The hardening experiments of HAR-

VEY (**10**), ROSA (**24**), and others also showed that the condition of hardiness may be rapidly established in plants under the influence of cool temperatures.

The writer has, so far at least, been unable to find any evidence of any appreciable loss of cold resistance by the leaves of the pitch pine during the winter months. In one experiment, branches of this species were brought into the greenhouse in February and kept with the cut ends in water for a period of three weeks. Parts of these branches were then exposed to a temperature of $-20°$ C. for several hours without any resultant injury to the leaves. Several similar experiments gave practically identical results. It appears possible that species may vary decidedly in the property of losing and regaining hardiness during the winter months. Perhaps this is related to a dormancy factor. The pitch pine is a species in which winter dormancy is very profound. However, the number of experiments which have been performed on this point is still limited, and the conclusion that warm weather will not diminish the cold resistance of pitch pine leaves during the winter months must still be regarded as tentative.

The work of HARVEY (**9, 10**), TUMANOV (**30**), and others indicates that the threshold temperature for inducing hardiness or cold resistance in plants is about 5°–6° C. HARVEY (**10**) found that exposure of cabbage plants for a period of one to four hours per day to a temperature of 0° C. kept these plants in a condition of hardiness, even if they were exposed to temperatures of 10°–20 °C. during the remainder of the day. This would appear to indicate, as he suggests, that as long as outdoor temperatures fall to a few degrees above zero for a short period each day, plants will remain resistant to injury at much lower temperatures. According to this conception, cold resistance would be induced in evergreen plants during the autumn months as soon as a fairly consecutive period of days occurred during which the temperature dropped close to 0° C. for a few hours each day. As late into the spring as such meteorological conditions persist plants would remain resistant to cold. Some species apparently may lose their cold resistance rather rapidly if a pronounced warm spell occurs during the winter months. Such species normally become hardy again as soon as exposed to hardening temperatures. On the other hand, there may be some plants which main-

tain their cold resistance, even through comparatively warm spells during the winter months. It is believed, as previously noted, that the pitch pine belongs in this class.

It is not proposed in the present paper to give any general discussion of the several important theories regarding the mechanism of low-temperature injury in plants, nor of the various theories of cold resistance in plants. Excellent résumés of the literature on these topics have been made by several previous investigators in this general field. The publications of CHANDLER (4), HARVEY (9), ROSA (24), NEWTON (21), HILDRETH (11), ÅKERMAN (1), and others contain excellent bibliographies of this subject. The most recent critical review of the work in this field is that of MAXIMOV (15).

The results of a previous investigation (17) upon the factors involved in the cold resistance of pitch pine leaves appeared to justify several general conclusions. No correlation could be found between the total water content of the leaves and the phenomenon of cold resistance in this species. The maximum difference between the water contents of hardened leaves during the winter and unhardened leaves during the following summer was found to be less than 2 per cent. Even when calculated in terms of hydration of the dry matter, this difference is relatively small. In actual fact the minimum leaf-water contents were found to occur in the late spring, at a period when the leaves of this species in Ohio are seldom subjected even to 0° C. temperatures, and never to appreciably lower ones. A marked accumulation of soluble carbohydrates was found to occur in the leaves during the winter months. Associated with this was an increase in the osmotic concentration of the expressed leaf sap of approximately equal magnitude. This led to the belief that the winter increase in osmotic concentration of the leaf cells is due largely to increase in soluble carbohydrates. Finally, on the basis of pressure dehydration experiments, it was concluded that an accumulation of colloidal water-imbibing substances occurred in the leaf cells of this species during the winter months, resulting in an increased imbibition pressure in the leaf cells. The increase in soluble carbohydrate content of the sap and the apparent increase in water-retaining capacity of the leaf tissues were considered to be the two most important internal conditions correlated with the property of cold resistance in

the leaves of this species. A correlation between accumulation of carbohydrates and cold resistance in plants has been traced by many previous investigators, and it is not the purpose of this paper to pay further attention to this phase of the problem of cold resistance. The investigations of ROSA (**24**), NEWTON (**20, 21**), MARTIN (**14**), STRAUSBAUGH (**27**), MEYER (**17**), and DUNN and BAKKE (**6**) especially have contributed to the conception that an increase in the proportion of water-retaining substances or of "bound" water in plant cells is an important factor in cold resistance. The contributions of these workers will be considered in more detail later in the discussion. In the present paper further consideration is given to the hypothesis that the accumulation of water-retaining colloids, or an increase in the bound (unfreezable) water, is an important factor in the cold resistance of the leaves of the pitch pine, and presumably of other evergreens.

Methods

The pine needles used in this investigation were obtained from native pitch pine trees which were growing on a hill slope in Fairfield County, Ohio, at a point about 35 miles southeast of Columbus. The pines in this location are about 20 years old and compose a rather open but otherwise typical "old-field" stand. For each set of determinations one branch was cut from each of eight different trees. Branches which were similar in exposure and general appearance were chosen. The material was collected late in the afternoon; after being transported to Columbus it was kept at out-of-doors temperatures overnight. The pine leaf samples were collected on August 25, 1931, and on January 15, 1932. The January collection was made just after a period of more than a week during which freezing or nearly freezing temperatures were attained every day. Even if the leaves of the pitch pine do lose their hardiness during winter warm spells, which seems improbable, conditions for some time just preceding this collection of leaves were favorable to the development of the hardened condition. For each collection determinations were made the next day.

Determinations were made on each of the eight branches of each collection of the total water content, resistance to dehydration under pressure, and amount of water which could not be frozen at -20° C.

(bound water). About 100 gm. of the 1931 crop of leaves were picked from each branch. All dead leaves and all dwarf branches were removed. Twenty gm. of each batch of leaves were used for the water-content determination. A second 20-gm. portion was used in the bound water determination, while about 50 gm. were used for the pressure dehydration determination.

The water-content determinations were made by drying the leaf samples to constant weight *in vacuo* at 70° C.

The determinations of the resistance of the leaves to expression of sap under pressure were made in a press chamber of a type described previously (**18**). The samples were first ground, and the ground sample weighed. Each was then subjected to a series of increasing pressures, ranging up to 3000 pounds per square inch, and the amount of sap which had been expressed when the tissue came to equilibrium with each pressure was recorded. From these data, and from the data on the total water content of the leaves, it is possible to calculate the hydration of the leaf tissue at each pressure and, if desired, to plot the actual pressure dehydration curves.

A determination of the amount of water in organic tissues which will not freeze at −20° C. has been considered by a number of investigators (**2, 8, 23, 25, 26, 29**) to be a measure of bound water. This quantity will be so referred to in this paper, although not necessarily with any specific implications regarding the nature of this bound water. Evidently, however, such water is in a special physical state; very commonly it is considered to be water which is held on surfaces by adsorption forces of sufficient magnitude to prevent the crystallization of water at this temperature. This unfreezable or so-called bound water may sometimes represent, in plant tissues at least, simply that portion of water in a solution which remains unfrozen at any given temperature below 0° C. At any temperature down to the eutectic point a certain portion of the water in a solution will remain liquid. This is due to the vapor pressure equilibrium which is maintained between the ice and the solution at temperatures below zero. As the temperature falls the concentration of the solution increases, owing to ice formation, until its vapor pressure equals that of the ice. This increase in the proportion of water in the frozen state continues until the eutectic point is reached, at which temperature the

solute crystallizes out and all of the water is converted into ice. Of course in solutions which are initially relatively dilute, the proportion of the water which remains liquid at $-20°$ C. is very small.

The amount of bound water was determined by the calorimetric method. Such a method apparently was first used, in a rather crude form, by MÜLLER-THURGAU (**19**). Later a much improved technique was proposed and employed by RUBNER. RUBNER's work became more widely known through the subsequent investigations of THOENES (**29**). Further refinements in this method have been introduced by ROBINSON (**23**), ST. JOHN (**25**), and SAYRE (**26**). The method about to be described, although modified to suit the present investigation, follows in general the work of these investigators. Since the experimental part of this work was completed, important papers dealing with certain theoretical aspects of bound water and its measurement by BRIGGS (**2**) and by JONES and GORTNER (**13**) have appeared. These papers will be referred to in more detail later in the discussion.

The pine leaves as used in the bound water determination were cut once into half, but otherwise were left intact. Each 20-gm. sample was inclosed in a brass freezing tube 1 inch in diameter and 6 inches in length. Each tube was stoppered at each end with a tight-fitting rubber stopper after the sample had been placed within. The stopper which was inserted in the top end of the tube carried a thermometer, set at such a level that its bulb was approximately in the center of the mass of leaves. These tubes were immersed vertically in an electrically refrigerated low temperature bath[3] in which a temperature of $-20°$ C. was maintained.

The freezing tubes were allowed to remain in this bath for several (3–4) hours, temperature equilibrium between the sample and the bath being reached some time before they were removed. Checks

[3] This bath was adapted from a General Electric water-cooler. The bottle was removed and the chamber of the cooler, which is about 10 inches deep and has an 8-inch top inside diameter, was nearly filled with 50 per cent alcohol. The switch was set so that the cooler would run continuously. It was supplied with a lid to support the tubes with the thermometers protruding, and a stirring device to keep the temperature uniform throughout the bath. This has proved to be an excellent device for small-scale experiments where temperatures down to $-20°$ C. are required. If necessary, such an arrangement can be provided with a thermostatically controlled heating element, but this was found to be unnecessary in the present work.

with different time intervals indicated that the freezing of water in the samples attained an equilibrium value in this period. BRIGGS (2) also records that with various organic colloids, as much ice formed at $-20°$ C. in 3–4 hours as in a week, indicating that equilibrium was attained in the shorter period of time. JONES and GORTNER (13) state that in a 50 per cent gelatin gel, freezing equilibrium is attained in less than one hour at $-21°$ C. At $-11.1°$ C., five hours were required for the attainment of this equilibrium, but just as much ice was formed at the lower as at the higher temperature. ST. JOHN (25) records that in the thick portion of egg white, freezing was complete in one hour at $-12.5°$ C. There seems to be no doubt that the technique used in the present investigations allowed the freezing equilibrium point to be attained.

A nickel-plated pint Thermos food jar, with a top opening 2 inches in diameter, was used as a calorimeter. Exactly 250 gm. of distilled water, warmed to about 3° C. above room temperature, were poured into the calorimeter. As soon as the water had come to a temperature equilibrium with the calorimeter walls, as indicated by the calorimeter thermometer, the determination proceeded. The calorimeter thermometer was graduated to 0.02° C., and had a temperature range of $+17°$ to $+32°$ C. All the other thermometers used in these determinations were calibrated against the calorimeter thermometer.

After the sample had come to equilibrium with the bath temperature, the brass freezing tube was removed from the freezing bath, quickly wiped dry, and the sample transferred to the water in the calorimeter. This was accomplished by removing the rubber stoppers from each end of the tube, and while holding one open end of the tube over the calorimeter, hitting the inclosed sample a sharp blow with a short wooden rod of slightly smaller diameter than the brass tube. No difficulty was encountered owing to sticking of the sample to the walls of the freezing tube. The entire operation of transferring the sample to the calorimeter was completed within a few seconds.

As soon as the sample was immersed in the water, the calorimeter was stoppered and allowed to stand for a short period (3 minutes). After this the entire contents were shaken by inverting and righting

the calorimeter three times in quick succession. The calorimeter thermometer was then inserted, being mounted in a stopper which fitted the mouth of the calorimeter snugly, and the temperature read as soon as the contents had come to equilibrium. After this the calorimeter was shaken again in the same manner and the final temperature redetermined as a check on the first temperature. This check reading never differed from the first reading by more than 0.05°, and usually an even closer check was obtained.

The calculations of the amount of bound water present are based on the following considerations. The loss of heat energy in terms of calories by the water in the calorimeter is represented by the quantity:

$$FNS_w(T-T_e),$$

where F represents the calorimeter factor, N the number of grams of water in the calorimeter, S_w the specific heat of water for the temperature range $(T-T_e)$, T the original temperature of the water, and T_e the equilibrium temperature.

The heat lost by the water in the calorimeter will be utilized in five different ways as follows: (1) to warm the dry matter in the sample from the original temperature of the sample to the equilibrium temperature; (2) to warm the ice in the sample from the original temperature of the sample to the melting point of the ice; (3) to warm the unfrozen (bound) water in the sample from the original temperature of the sample to the melting point of the ice; (4) to warm *all* the water in the sample from the melting point of the ice to the equilibrium temperature; and (5) to melt the ice.

Each of these quantities is expressed mathematically as follows:

(1) $W_dS_d(T_e-T_s)$
(2) $W_iS_i(T_m-T_s)$
(3) $W_bS_b(T_m-T_s)$
(4) $W_wS_w(T_e-T_m)$
(5) QW_f

Where:

W_d = weight of dry matter in the sample;
W_b = weight of bound (unfrozen) water in the sample;
$W_i = W_f$ = weight of ice (free water) in the sample;

W_w = weight of all water in the sample;
S_d = mean specific heat of dry matter for the temperature range indicated;
S_i = mean specific heat of ice for the temperature range indicated;
S_b = mean specific heat of bound water for the temperature range indicated;
S_w = mean specific heat of water for temperature range indicated;
T_s = temperature of sample;
T_m = melting point of ice in the tissue = freezing point of water in the tissue;
T_e = equilibrium temperature;
Q = heat of fusion of ice at T_m.

It is evident that the sum of these five quantities is equal to the quantity:

$$FNS_w (T - T_e).$$

Equating, and solving for W_f, remembering that $W_b = W_w - W_f$, the following equation is derived:

$$W_f = \frac{FNS_w(T - T_e) - [W_d S_d(T_e - T_s) + W_w S_w(T_e - T_s)]}{Q - [(S_b - S_i)(T_m - T_s)]}.$$

The values for T_s and T_m, being below zero, should be substituted in the preceding equation with a negative sign. By subtracting the amount of free water as calculated by the equation from the total water, the quantity of bound water present is determined.

The arguments followed in deriving the preceding equation follow in the main those found in the papers of THOENES (**29**) and ROBINSON (**23**). Their equations are also given by GORTNER (**8**). In the papers of ST. JOHN (**25**) and SAYRE (**26**), both of which appeared after the experimental part of this work was completed, the same general line of reasoning was followed. The final equation as here presented takes a slightly different, and in some respects it is believed, an improved form from that given by any of the aforementioned workers.

A correction factor for the calorimeter is necessary because a small

portion of heat lost by the water goes into the warming of the walls of the calorimeter and the thermometer instead of into the plant tissue. This factor was determined by introducing into the calorimeter masses of ice of known weight and of as nearly the same volume as possible as a 20-gm. sample of the pine leaves. Exactly the same procedure was followed as when determinations were made with the pine leaves. Since the number of calories required to change the temperature of ice and water and to melt the ice is known, a correction factor could readily be calculated from the values obtained for the change in temperature of the water. The factor for the system used in this work was found to be 1.06, this being the average value obtained from a number of closely agreeing determinations.

The specific heat of the dry matter of the pine needles was also determined in the calorimeter, following exactly the procedure previously outlined, except that benzene was substituted as the calorimeter liquid instead of water. Because of the high specific heat of water, it was found that the temperature changes when dry plant material was used were so small that the chances for errors were greatly enhanced. The use of a liquid of lower specific heat increased the probable accuracy of the determinations. The specific heats for benzene as given in the International Critical Tables (**31**) were used in these calculations. Obviously the calculation is simplified when a dry material is used, as the heat lost by calorimeter liquid goes only to warm up the dry material. The specific heat of the dry matter of pine needles was found by this method to be 0.30 calories.

The values used for the specific heat of water above zero are those given in the tables of the Handbook of Physics and Chemistry (**12**). The values used for the specific heat of water below zero were estimated by extrapolating the curve for specific heats of water as given in these same tables for values from $-6°$ to $-20°$ C. The values for the specific heat of ice and for the heat of fusion of ice are those given in the tables of DICKINSON and OSBORNE (**5**). SAYRE (**26**) gives a convenient summary table of the specific heats of ice and water, based on the same sources as used in this paper. The specific heat for bound water has been assumed to be the same as that of free water at the same temperature. The freezing (melting) temperature of the free water in the pine needles was assumed to be equal to the

freezing-point depression of the expressed sap. That this is probably not a strictly correct assumption is indicated by the work of CARRICK (3), who showed that the actual freezing temperature of the tissues of entire apple fruits is slightly lower than the freezing point of the sap expressed from the same organs. The same is presumably true for other tissues. The error involved is small, however, seldom exceeding 1° C., and may be disregarded without any appreciable effect on the results.

Results and discussion

The results of the pressure dehydration experiments are presented in table I, in terms of the percentage hydration of the dry matter of the ground pine leaves when in equilibrium with each of the series of pressures used. The values for each of the eight individual determinations as well as the mean for each series are tabulated. The initial percentage hydration is much higher in the August series, owing of course to the greater water content of the tissues at that season. It is also possible to express a significantly larger amount of water from the tissues in the summer than in the winter. This, however, as is evident from the other figures in this table, is also due principally to the greater water content of the tissues at this season.

At all of the pressures used, the mean hydration percentages are slightly higher in the January series as compared with the August series. Comparison of these means was made by FISHER'S method (7) of testing the significance of the difference of two means. The odds were only slightly in favor of any of these differences being significant; hence this method fails to demonstrate any unquestionable difference in the water-holding capacity of the leaf tissues in the winter as compared with the summer.

In a previous investigation (17), the writer found that pine leaves frozen at −20° C. in December showed a markedly greater resistance to dehydration under pressure than other samples of the same crop of leaves similarly treated the preceding August or the following June. This result, however, must be regarded as primarily due to the fact that freezing of the leaves in the summer resulted in death of most or all of the cells present, while in December little or no injury resulted. Such a determination is a measure of whether or not the

leaves have been injured by the temperatures to which they have been exposed more nearly than anything else. Similar, but much smaller differences were obtained with intact unfrozen leaves when they were subjected to pressure on the same dates. This seemed to indicate at that time an increased imbibitional pressure of the leaves in

TABLE I

PERCENTAGE HYDRATIONS OF GROUND LEAF SAMPLES

SAMPLE	PERCENTAGE WATER CONTENT	SAP EXPRESSED PER 100 GM. TISSUE	PERCENTAGE HYDRATIONS OF DRY SUBSTANCE AT INDICATED PRESSURES (POUNDS PER SQUARE INCH)					
			0	375	750	1500	2000	3000
	August 25, 1931							
1.....	63.4	46.2	174.0	68.0	61.8	54.5	51.1	47.7
2.....	63.1	41.9	170.0	73.4	67.7	63.3	59.4	56.9
3.....	62.5	41.2	166.7	73.7	67.5	62.5	60.0	56.9
4.....	62.8	42.3	170.0	75.8	66.0	60.9	57.1	55.3
5.....	63.2	43.6	174.0	70.6	65.9	59.7	56.5	54.4
6.....	62.5	44.2	166.7	63.8	57.1	52.9	50.9	48.6
7.....	60.1	42.1	150.0	59.9	55.5	49.8	47.4	44.9
8.....	60.1	38.9	150.0	67.8	61.3	59.5	55.3	53.0
Mean..	62.2	42.6	165.1	69.1	62.8	57.8	54.7	52.2
	January 15, 1932							
1.....	58.3	34.4	141.0	71.0	67.4	63.3	58.9	58.0
2.....	57.5	30.8	135.2	75.7	71.1	66.4	64.2	63.4
3.....	57.2	33.7	132.5	68.5	64.0	58.3	56.3	53.8
4.....	57.7	32.2	138.1	75.3	69.8	66.4	64.3	61.5
5.....	57.4	37.1	135.2	59.2	54.0	52.6	50.6	49.0
6.....	56.6	32.2	129.9	70.6	65.3	60.9	57.8	56.1
7.....	57.1	33.1	132.5	68.2	64.2	58.2	56.7	55.2
8.....	56.8	33.8	129.9	64.8	60.0	56.7	54.1	52.5
Mean..	57.3	33.4	134.3	69.2	64.4	60.4	57.8	56.2

the winter as compared with the summer, but it now appears doubtful whether these results were actually of significance.

In contrast with the previous investigations in which intact leaves were used, the needles as used in this work were first coarsely ground before they were subjected to pressure. This was done to insure a more homogeneous packing of the press chamber and consequently a more equable distribution of pressure throughout the sample un-

dergoing pressure. If any significant difference in the imbibitional capacity of the cell colloids were present at different seasons of the year, it seemed probable that this technique would come closer to giving reliable indications of it than any other. As the previously presented data show, no indication of any appreciable difference has been found.

NEWTON (**20**), however, who was the first to use such a method as a measure or indication of cold resistance, was able to show rather marked differences in the hydration which would be maintained by the leaves of hardened wheat plants when placed under pressure as compared with the leaves of unhardened plants. The hardened leaves always maintained a higher hydration than the unhardened leaves at the same pressure, in spite of the fact that the initial water content of the unhardened leaves was always greater. These results were obtained with intact leaves, untreated in any way.

MARTIN (**14**), working subsequently, was able in general to confirm NEWTON'S results with wheat plants. The volume of press juice expressed usually showed an inverse correlation with the relative hardiness of the variety the leaves of which were subjected to pressure. Such results were obtained with both frozen and unfrozen leaves, although a number of irregularities occurred. For reasons mentioned in a preceding paragraph, it appears to the writer that the results of pressure-dehydration determinations made with unfrozen leaves are much more likely to be significant than those made with frozen leaves.

STEINMETZ (**28**) also employed this method on alfalfa roots, but could find no correlation between the volume of juice expressed and hardiness. Since he first froze his samples at a low temperature, however, thus probably killing all or most of the cells, such a result is not surprising.

The results of the calorimetric determinations on the 1931 leaves of the pitch pine in August, 1931, and January, 1932 are presented in table II. Eight separate samples were determined from collections on each of these dates, each sample being from a different tree. Data are given for the total water, dry matter, free water, free water per gram of dry matter, bound water, and bound water per gram of dry matter for each, as well as the mean for each set of eight figures.

The mean value for each of the August determinations was compared with the corresponding mean as determined in January, by FISHER's method of testing the significance of the difference between two means. The odds were overwhelming that there was a signifi-

TABLE II

RESULTS OF CALORIMETRIC DETERMINATIONS OF BOUND AND FREE WATER IN PINE NEEDLES

SAMPLE (20 GM.)	WATER (GM.)	DRY MATTER (GM.)	FREE WATER (GM.)	FREE WATER PER GM. DRY MATTER (MG.)	BOUND WATER (GM.)	BOUND WATER PER GM. DRY MATTER (MG.)
	August 25, 1931					
1	12.67	7.33	9.33	1272	3.34	456
2	12.63	7.37	9.07	1231	3.56	483
3	12.50	7.50	9.14	1219	3.36	448
4	12.54	7.46	9.47	1269	3.07	411
5	12.64	7.36	9.23	1254	3.41	463
6	12.51	7.49	9.41	1256	3.10	413
7	12.08	7.92	9.03	1140	3.05	385
8	12.05	7.95	8.91	1120	3.14	395
Mean	12.45	7.55	9.20	1220	3.26	432
	January 15, 1932					
1	11.66	8.34	8.60	1031	3.06	367
2	11.49	8.51	7.82	919	3.67	431
3	11.44	8.56	8.13	950	3.31	387
4	11.54	8.46	8.02	948	3.52	411
5	11.48	8.52	8.51	999	2.97	349
6	11.31	8.69	8.12	934	3.19	363
7	11.42	8.58	8.40	979	3.02	353
8	11.38	8.62	8.21	952	3.17	368
Mean	11.46	8.54	8.23	964	3.24	379

cant difference between every pair of means, except the two for the total amount of bound water present.

The total water content of the samples is less in winter than in summer, the dry matter content showing a reciprocal relation of course. This decrease in water content in passing from the summer to the winter condition has been observed in this species during a number of previous years. This means also, as shown in table I, that

the total hydration of the tissues is less in the winter than in the summer.

The quantity of free water present is larger in the summer than in the winter. This difference is further emphasized when the calculations are made on the basis of a unit of dry matter, which reduces the values to a more nearly comparable basis in terms of the actual substance involved. The higher free-water content in the summer appears to bear a close correlation with the higher total hydration of the leaf tissues at that season. That is, with decrease in the total hydration of the leaf tissues there is a decrease in the amount of free water present, and vice versa.

The total quantity of bound water present in the winter is not significantly different from the quantity present in the summer. Calculations based on a unit of dry matter, however, indicate a significantly greater amount of bound water in the summer as compared with the winter. Data regarding the total amount or percentage of bound water in any colloidal system in themselves reveal very little about the physico-chemical properties of that system; only when such data are referred to the dry matter content do they become intelligible. Although the total amount of ice formed in equal weights of leaves is greater in the summer than in the winter, the residual hydration of the dry matter after freezing of the leaf tissues is greater in the summer than in the winter. Decrease in the total hydration of the system appears to result in a decrease in the amount of bound water as well as of free water.

During the year 1930–1931, a similar series of bound-water determinations was made on the 1930 leaves of the pitch pine. These results are not reported in the present paper because the technique employed in obtaining them was not quite up to the standards adopted for the 1931–1932 series. The results were concordant with those under discussion, however, in that they also indicated a higher proportion of bound water per gram of dry matter in the summer than in the winter.

As-yet unpublished work from this laboratory by Miss CHRYSLER on the amounts of bound and free water in a plant colloid (the stipe of the Elk kelp, *Pelagophycus porra*) at different degrees of hydration has resulted in substantially similar conclusions. These results

are also supported by data on various colloidal systems by NEWTON and MARTIN (**22**), BRIGGS (**2**), and JONES and GORTNER (**13**). In general, therefore, it appears that the amounts of bound and free water in a colloidal system are positively correlated with the total degree of hydration of the system. This is, in fact, what would be expected if the bound and free water in a system are considered to be in equilibrium. Plant tissues, the present work shows, are no exception to this principle, and for the purpose of such a conception may be regarded as representing exceedingly complex, hydrated colloidal systems.

These results conflict with those of several preceding investigators who have used a measure of the unfreezable water in plant tissues as indication of the amount of bound water present, and whose work indicates a correlation between the amount of bound water present and cold resistance.

ROSA (**24**) appears to have been the first to present experimental data which favored the view that increasing hardiness is correlated with an increase in the bound water content of the tissues. He used cabbage plants in most of his experiments, employing the dilatometer method as developed by MCCOOL and MILLAR (**16**) for determining the amount of water which would freeze. The percentage of the total water freezing at temperatures down to $-6°$ C. was found to be less in hardened cabbage plants at every temperature than in unhardened plants. Conversely, the total amount of water remaining unfrozen was greater at every temperature in the hardened as compared with the unhardened plants. Furthermore, it was found that the percentage of the total water in cabbage plants which could be frozen at $-5°$ C. decreased progressively in the period of 20 days during which the plants were being hardened in a cold frame.

At first glance it would appear that these results are completely at variance with those reported in the present paper. Further consideration shows, however, that the discrepancies are not so great as they appear. ROSA's results are presented in terms of grams of water remaining unfrozen in 100 gm. of tissue. Since during the hardening of the cabbage plants there was always an increase in dry weight, the calculation of his results in terms of the grams of water remaining unfrozen per gram of dry weight markedly reduces the increase in

bound water found in hardened leaves as compared with unhardened ones. As previously pointed out, this is probably the most significant terminology in which to express the amounts of bound water present. Furthermore, the lowest temperatures used by ROSA were $-5°$ and $-6°$ C., instead of $-20°$ C. as used in the present work. It seems probable that had such a lower temperature been used, the differences found in the amounts of bound as compared with free water might have been still further reduced. This seems especially true, since ROSA allowed only a very short time for equilibrium to become established in his determinations. JONES and GORTNER (**13**) have shown in their experiments that a relatively long period is required for freezing in organic colloids to become complete at a temperature of $-11.1°$ C., while equilibrium is usually attained much more rapidly at a temperature of $-20°$ C. Furthermore, although in some colloidal systems (**13**) as much water freezes at $-6°$ C. as at $-20°$ C., if sufficient time is allowed for the attainment of equilibrium, this does not prove that the same is true for plant tissues in which the liquid medium is not water, but a solution sometimes of considerable concentration.

Finally there is the possibility that this increase in unfreezable water is due, in part at least, simply to the increase in soluble carbohydrates, which, although not great, is shown to occur during the hardening of cabbage plants by ROSA'S own analyses. MAXIMOV (**15**), CHANDLER (**4**), and ÅKERMAN (**1**) especially have championed the conception that the higher the concentration of soluble carbohydrates in a plant cell the smaller the proportion of water which will freeze at any given temperature below 0° C.

In a molar solution of sucrose, for example, it is known that about 50 per cent of the water will freeze at about $-4.336°$ C. (the freezing point depression of a 2M sucrose solution according to BRIGGS, **2**), while only traces of ice will form in a 2M solution at the same temperature. The possible relation of this to cold resistance is elaborated in the papers of the three investigators just mentioned. Other solutes would be equally effective, providing only that their eutectic points are below that of the freezing temperature. Although this is a well known physico-chemical fact, and there seems no reason to believe that cell sap solutions would not behave in the same way, the direct

evidence from plants on this point is still fragmentary. Nevertheless this remains an important possibility, and it seems likely that some of the increase in bound water which occurs in hardened plants is simply due to the effects of the increased soluble carbohydrate content of the cell sap, which is an almost invariable accompaniment of an increase in hardiness.

ROBINSON (**23**) has made an investigation of the relation of bound water to the hardening process in insects. The calorimeter method was used, the exact technique being similar to that followed in the present investigation. When the larvae of the Promethea moth (*Callosamia promethea*) were gradually cooled in refrigerating cabinets from 18° C. (temperature of the cabinets) until the internal temperature of the insects was reduced to −6.5° C. at a cabinet temperature of −14° C., a marked increase in the amount of bound water present at the expense of the free water occurred. Maximum values for bound water were attained in about two weeks' time. When similar experiments were conducted with non-hardy species, either there was a very slight increase in bound water or else an actual decrease.

Attention should be called to the fact that the term "hardening" as used by ROBINSON with reference to insect larvae does not have the same meaning as the definition adopted for the term in this paper. His experiments were conducted at temperatures above the actual freezing points of the larvae, so that ice did not form in the tissues at any time, except during the calorimetric determinations. In the pitch pine and other species to which this paper has special reference ice often forms in the tissues. There are, as is well known, hardy and non-hardy plants with respect to the range of low temperatures just above the point of actual ice formation in the tissues, but it is not hardiness of this type which is at present under consideration. There are also species of insects the larvae of which will survive the formation of ice in the tissues, but ROBINSON's work did not deal with such conditions.

These results do not agree with those reported for pitch pine in the present paper, and only further investigation will eliminate or explain the discrepancies. It is possible that bound water may play a more important rôle in the hardening of insect larvae than in the hardening of plants. ROBINSON believed that the increase in bound

water as observed in his experiments was due principally to an increased adsorption of water, presumably on colloidal surfaces, at the lower temperatures. It is possible that a gradual cooling of living tissues over a period of a week or more, as in ROBINSON'S experiments, might cause a shift in the bound-free water equilibrium toward the bound water side. The natural conditions to which the pitch pine leaves had been exposed prior to the winter determinations recorded in this paper were not identical with the conditions employed by ROBINSON. The pine leaves, however, had just been exposed to a period of more than a week of cool temperatures, and were unquestionably in the hardened condition, although the temperatures to which they were exposed were intermittent and not as low as those in ROBINSON'S experiments.

There is another important difference between the conditions in ROBINSON'S experiments and those reported in the present paper. The insect larvae with which he worked showed very little variation in total water content, while the hardened pitch pine leaves had a distinctly lower water content than the unhardened leaves. As pointed out previously, the amount of bound water in the leaves of these species appears to correlate directly with the total hydration of the leaves. Any effect which lowering of the temperature might have in increasing the amount of bound water, or adsorbed water, may be obscured in the pitch pine leaves by the decreasing effect of a lower total hydration of the leaves. Experiments are projected to test the exact effects of controlled changes in temperature upon the amount of bound water in pitch pine leaves under such conditions that little or no change in the total hydration of the tissues occurs.

Another difficulty arises in attempts to compare bound-water values obtained for plant tissues with those obtained for animal tissues, owing to the much more prominent rôle played by the cell wall in plants. NOVIKOV, as cited by MAXIMOV (**15**), found that wheat leaves which had been killed with chloroform gave the same values for bound water determinations as living leaves. This and other considerations suggest that the great bulk of the bound water in plant tissues may be in the cell walls. A very large proportion of the dry matter of leaf tissues is to be found in the cell walls; much of this substance is hydrophilic; and theoretically it would be expected to con-

tain considerable quantities of water in that physical condition usually spoken of as bound water. This conception would relegate the protoplasm, and cell contents generally, to a relatively minor part in the binding of water in plant tissues, and helps clarify the similarity in behavior between non-living colloidal systems and living tissues with respect to the effect of the total hydration of the system on the amounts of bound and free water present. The smaller the quantities of solutes present, with their colligative effects on the amount of water freezing in a system, the closer the similarity which might be expected between living and non-living systems.

It is evident that the results of this investigation offer no support for the hypothesis that the binding of water by colloids is an important factor in the cold resistance of the pitch pine. Presumably the same is true for the leaves of other evergreens. This does not eliminate the possibility, of course, that colloids play a rôle of importance in the phenomenon of cold resistance. They may operate through some other mechanism than that of preventing the freezing of water. It is conceivable that there may be increases in the actual quantities of material in the colloidal condition, or even sol to gel transformation, without any marked effect on the amount of bound water present. Certain types of colloids might operate to stabilize the protoplasm against the desiccating effects of freezing. Stabilization of inorganic sols in this way can readily be demonstrated. Another possibility is that changes in the colloidal organization of the cell contents might work in such a way that the size of the ice crystals or mode of crystallization might be affected.

In the last analysis it seems clear that no physico-chemical condition has been discovered which is an invariable accompaniment of the state of cold resistance in plant cells. Some conditions, such as an increased sugar content of the cells, frequently correlate with the ability of plant cells to survive exposure to low temperatures; but there are always important exceptions. None of these correlating factors is definitely known to be causatively related to cold resistance. It seems probable that the ultimate basis for this phenomenon lies in the protoplasm itself, and that attempts to resolve the problem by gross physiological measurements are doomed to be unsuccessful. Except in tissues of very low water content, in which the

protoplasm is initially at a low hydration percentage, exposure of plant cells to low temperatures apparently always results in a more or less severe desiccation of the cells owing to the conversion of a large portion of the water present into ice. The severity of this desiccation depends to some extent upon the temperature to which the cells are subjected, the duration of the exposure, and, for any given species, upon the exact physiological conditions which prevail in the cells at the time of freezing. It seems probable, therefore, that the ultimate consideration in hardiness and cold-resistance phenomena is whether or not the protoplasm itself can endure this desiccation. Protoplasms which can endure desiccation of this type will survive low temperatures without destruction; those which cannot will be destroyed or severely injured. In many species the same protoplasmic units may at times be able to survive the condition, while at other times they cannot. In the last analysis, the basis of cold resistance is almost certainly to be found in the physico-chemical organization of the protoplasm itself. Until more precise methods of investigating protoplasm from this standpoint are devised, it appears that further progress in the problem will be very slow. MAXIMOV (**15**) considers the basic factor in drought resistance to be the ability of the protoplasm to endure periods of desiccation, due to loss of water, without injury. There appear to be some marked similarities in the physiological mechanisms which lie at the basis of these two properties of plants. This analogy, although enticing, must not be pushed too far, as curiously enough there are many plants the protoplasm of which can endure true desiccation as engendered by drought conditions, but cannot endure the physiological desiccation caused by ice-crystal formation in the tissues.

Summary

1. Seasonal variations in the cold resistance of the leaves of evergreen species are discussed in terms of the present knowledge of such phenomena. Evergreen leaves, in general, are not cold-resistant during the warmer months of the year. Time and temperature factors involved in the hardening of the leaves of such species are analyzed in terms of known facts. The work of several investigators indicates that the threshold temperature for hardening seems to be about

+6° C. A few hours' exposure to this or a lower temperature per day suffices to keep the leaves in the hardened condition. During warm spells in the winter the leaves of some species of plants seem to lose their hardiness very quickly; in other species the physiological changes causing loss of hardiness appear to occur much more slowly.

2. In this investigation attention was directed to further studies bearing on the hypothesis that an increase in bound water is an important factor in the cold resistance of the leaves of evergreen species. A pressure dehydration method of distinguishing between bound and free water in plant tissues is outlined. A modified form of the calorimetric procedure of determining the amount of free water in a system, suitable for use with plant tissues, is described. An improved form of the equation for calculating the amount of free water from the calorimetric data is also derived.

3. The leaves of the pitch pine (*Pinus rigida* Mill.) were used as the material for investigation. The total amount of water in the leaves of this species was about 5 per cent less in the winter as compared with the summer. Larger amounts of sap can be expressed from these leaves in the summer than in the winter, but this difference represents almost entirely a correlation with the difference in total water content. No evidence could be obtained by the pressure dehydration method of any significant increase in the amount of bound water in the pine leaf tissues in the winter as compared with the summer. The calorimetric determinations showed greater amounts of both bound and free water per gram of dry matter in the leaves of this species in the summer as compared with the winter. The differences between the summer and winter values were statistically significant. Apparently, as has been found previously in non-living colloidal systems, there is a positive correlation between the total hydration of the tissues and the amounts of both bound and free water per gram of dry matter. No evidence was obtained, therefore, that increase in bound water plays any rôle in the cold resistance of this species.

4. The results of other investigations are critically analyzed in relation to the results of this investigation. It is suggested that the basis for cold resistance lies in some as yet not understood physico-chemical properties of the protoplasm, which probably cannot be

discovered by the gross measurements which are generally employed at present.

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CYTOLOGY OF ANTENNARIA[1]

II. PARTHENOGENETIC SPECIES

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(WITH PLATES II, III AND THIRTEEN FIGURES)

The purpose of this investigation, as well as the history of the subject and the material and methods, was outlined in a previous paper (STEBBINS **38**).

Observations

DEVELOPMENT IN STAMINATE FLOWERS

In the seven species of *Antennaria* here studied, which reproduce entirely (or almost entirely) parthenogenetically, the staminate plants are rare or unknown. In only three could material for a study of the microsporogenesis be obtained.

A. fallax Greene, staminate material from a dry, scantily wooded knoll near Canton Junction.—The plant is larger in all its parts than *A. plantaginifolia*, its nearest relative, and the cells are all of considerably greater size. At diakinesis both paired bivalent chromosomes and unpaired univalents are present. At the heterotypic metaphase the bivalent chromosomes are arranged at the equatorial plate, while from two to twelve univalents are scattered over the spindle (fig. 14). Enough counts were made on favorable metaphase cell plates to fix the haploid number at forty-two. Figure 15 shows a metaphase cell plate at which forty pairs of bivalent and four univalent chromosomes can be counted. The univalents can be recognized from their lesser depth of focus, and they are frequently at a different plane of focus from the cell plate.

The univalents lag near the center of the spindle, or are scattered in the cytoplasm during the heterotypic anaphase, and generally form one or two extra nuclei during interkinesis. The homoeotypic division is for the most part regular, and figure 17 shows a homoeotypic metaphase in which 41 chromosomes may be counted in one

[1] Contribution from the Laboratory of Plant Morphology, Harvard University.

cell plate, one chromosome having been ejected into the cytoplasm. There are often extra nuclei at the homoeotypic telophase, and about 60 per cent of the "tetrads" are of the polysporic type. The pollen is about 50 per cent perfect.

Cytomixis was frequently observed in *A. fallax*, mostly during the prophase. Figure 16, however, shows a heterotypic anaphase where cytoplasmic connections with a neighboring cell have persisted. Figure 18 shows a homoeotypic telophase in which two of the nuclei of one cell are stretched across and invade the cytoplasm of a neighboring cell. Both cells show signs of shrinkage and degeneration, but neighboring cells in the same anther loculus were perfectly normal in appearance.

The staminate plants of *A. fallax* studied are of the "aberrant male" type described by JUEL (**21**) in *A. alpina*. The flower heads are higher than those of pure staminate flowers, the pappus hairs have only slightly dilated tips, the style is longer than in typical staminate flowers, while the stigma has two short branches instead of a single capitate tip. Achenes develop in 2 to 25 per cent of the flowers in each head, the average being about 10 per cent. In these achenes, a megaspore mother cell may be seen, first in the spireme stage, and later in diakinesis. At the heterotypic metaphase, there are bivalents at the equatorial plate, while a large number of univalents are scattered about the spindle (fig. 36). The heterotypic division is almost regular; and, although no chromosome counts could be obtained at any stage, owing to scarcity of material, reduction appears to have taken place (fig. 37). However, fusion may possibly take place, after the homoeotypic division, between the two daughter cells of the spindle at the chalazal end of the nucellus. The only megaspore group observed contained only three large nuclei, that at the chalazal end being distinctly larger than the other two (fig. 38). The development of the embryo sac, owing to scarcity of material, could not be perfectly studied.

A. parlinii Fernald, staminate material from dry field, Ballston, Virginia.—The flowers are of the aberrant male type, although the proportion of developed achenes is smaller than in *A. fallax*. No stages of meiosis in the achenes were found.

In the pollen mother cells there are univalents, bivalents, and pos-

sibly trivalents and quadrivalents at diakinesis. At the heterotypic metaphase from ten to sixteen univalent and loosely paired chromosomes are scattered over the spindle (fig. 19). In polar view, the chromosomes are clumped together in groups so that counting is difficult. Counts ranged from 39 to 46 as the haploid number; but in the most favorable cells a haploid number of 42 was more or less clearly seen. Figure 20 shows such a cell, with 37 bivalents and 10 univalents. Here most of the bivalent pairs are grouped into quadrivalent and sexivalent associations. There is more lagging during the heterotypic division than in *A. fallax*, and 90 per cent of the "tetrads" are polysporic (fig. 22).

Rarely the heterotypic division fails of completion, and a "restitution nucleus" results. This nucleus is most often dumb-bell shaped, but may be irregular in form, as in figure 21. This restitution nucleus evidently divides with a division of the homoeotypic order, resulting in a dyad of microsporocytes, each of which has the diploid number of chromosomes (fig. 23).

A. canadensis Greene.—The following staminate plants were studied: no. 540, grassy hillside, Lexington. Leaves not so bright green as in typical *A. canadensis;* stolons assurgent; cauline leaves with scarious appendages, although these are not so well developed as in typical *A. canadensis*. Although this plant shows some transitions toward *A. neodioica*, all the pistillate plants found in the immediate vicinity of its locality were typical *A. canadensis*. Flowers were of the aberrant male type. No. 757; Canton Junction. The plants correspond in every respect to typical *A. canadensis*. Flowers were of the pure male type.

In no. 540, the diakinesis stage was not often found. At the heterotypic metaphase there are generally a few paired bivalents and a large number of univalents. At the heterotypic anaphase the bivalent pairs split and move toward the poles, so that the chromosomes are scattered irregularly over the spindle. In favorable spindles, at this stage, approximate counts can be made, although the large number precludes absolute accuracy. Figure 24 shows 83 chromosomes, which is about the diploid number for *A. canadensis*. This stage is found very frequently, and apparently lasts for a long time. The spindle becomes gradually narrower and the chromosomes more

clumped together, while the spindle may become bent, as in figure 25. The subsequent development occurs in one of two ways:

1. The chromosomes may all, or nearly all, reach the poles, and be inclosed in the daughter nuclei at the heterotypic telophase. Here the homoeotypic division proceeds regularly, and results in a tetrad or polysporic group. Figure 31 shows a homoeotypic metaphase cell plate, where 40–42 chromosomes, the haploid number, can be counted.

2. The heterotypic telophase may set in before the chromosomes have reached the poles, and a restitution nucleus may thus be formed (fig. 26). The chromosomes then go through the interkinesis stage, apparently losing their identity, while a nucleolus frequently appears. Later they are seen in the prophase of the homoeotypic division, appearing longer and more slender than in the heterotypic division, and many show clear evidence of splitting (fig. 27).

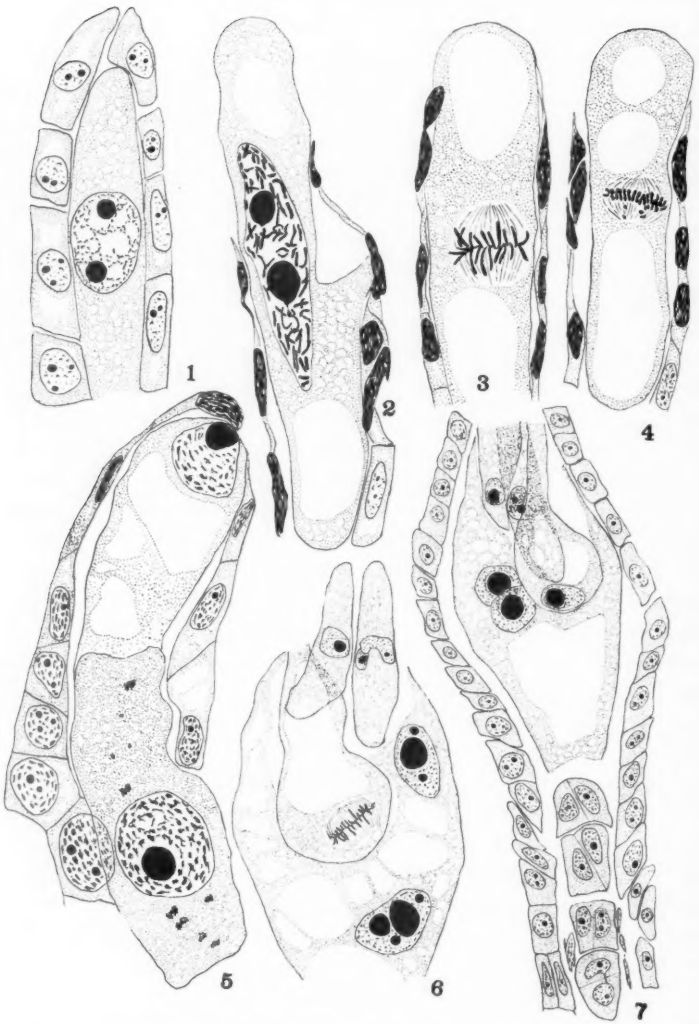
A homoeotypic, equational division then takes place, at which there is only one spindle (fig. 28). Figure 29 shows the polar view of such a spindle, at which about 86 chromosomes can be counted, clearly the diploid number. The division proceeds for the most part regularly, although figure 30 shows a telophase at which lagging chromosomes have formed a bridge between the daughter nuclei. The result is a dyad, although one or more microcytes may be present.

The percentage of occurrence of these types of development is indicated by the relative abundance of the various types of microspore groups, which is: dyads 27, tetrads 34, polysporic "tetrads" 39 per cent. Many of the apparently normal tetrads must later degenerate, as practically all of the mature pollen grains are evacuolate or otherwise abnormal in appearance.

In no. 757 the development is much the same, and the chromosome number, although it could not be counted accurately, is about the same. The percentage of the various types of microspore groups is: dyads 37, tetrads 8, polysporic groups 55 per cent.

Development in pistillate flowers

In all of the seven parthenogenetic species there are two types of development in the pistillate flowers, a general description of which will, with slight deviations, apply to all.



FIGS. 1–7.—Fig. 1, *A. fallax*, embryo sac mother cell. Fig. 2, *A. petaloidea*, prophase, first embryo sac division. Fig. 3, *A. canadensis*, normal metaphase of same. Fig. 4, *A. canadensis*, no. 532, abnormal metaphase of same. Fig. 5. *A. parlinii*, archesporial cell replaced by integumentary one. Fig. 6, *A. petaloidea*, first division of egg nucleus. Fig. 7, mature embryo sac. Figs. 1–5, ×1200; figs. 6, 7, ×600.

The first is the type described by JUEL (**21**) for *A. alpina*, and designated by ROSENBERG (**35**) as the *Antennaria* type. Here the young megaspore mother cell does not show a definite spireme stage. At the age in which the spireme is seen in the megaspore mother cells of *A. plantaginifolia*, and of the "aberrant males" of *A. fallax*, there are frequently a few thin threads formed, but these soon break up again into chromatin granules (fig. 1), and soon these granules are scattered at random throughout the nucleus. At all stages the nucleus stains weakly, except for the nucleoli. As the young embryo sac increases in length, the nucleus increases in size but does not divide. The formation of a tetrad of megaspores is entirely omitted. As the nucellar cells start to degenerate, the nucleus moves out to the micropylar end of the young embryo sac, and a vacuole forms at the chalazal end. When most of the nucellar cells have degenerated and the embryo sac has pushed out beyond them, this mother nucleus divides for the first time. This division is carried through very quickly, and its stages are difficult to find. At the later prophase the nucleus is often very much elongated, and there are a large number of long, slender, unpaired chromosomes (fig. 2). This resembles closely the similar stage in *A. alpina* and in *Taraxacum albidum* (OSAWA **27**). An interesting feature in figure 2 is the very clear budding of the lower nucleolus, a phenomenon seen only in this instance. At the metaphase the chromosomes are all of the long, slender, somatic type, and are arranged as in an ordinary somatic division (fig. 3). The daughter cells resulting from this division migrate to either end of the embryo sac, and there undergo the two somatic divisions which result in the young 8-celled embryo sac. It was impossible to obtain chromosome counts at any of these divisions, owing to the large number and the interlocking of the very long and slender chromosomes; but in *A. fallax*, *A. petaloidea*, and *A. canadensis* the number was definitely determined as greater than the haploid number for the species.

The embryo sac grows into the usual flask shape, although the polar nuclei do not fuse, but lie next to each other against the egg cell until just before the endosperm begins to develop (fig. 7). The number of antipodals is only eight to ten, less than that found in the mature embryo sac of the non-parthenogenetic species (STEBBINS **38**).

In figure 7, the two antipodal nuclei at the chalazal end of the group are connected through the cell membrane separating them by a thread of nuclear material. This was probably caused by the accidental lagging of one of the chromosomes during the late anaphase of the division to form these nuclei. Similar threads connecting antipodal nuclei, where, however, no cell membrane separated them, were found in *Artemisia maritima* by WEINEDEL-LIEBAU (41), and interpreted as evidence of amitosis. It is doubtful, however, that such has taken place here.

The first division of the egg cell takes place when the endosperm is 2- or 4-nucleate (fig. 6). The resulting daughter nuclei are separated by a horizontal wall, and that nearest the micropyle fails to divide further. The next few divisions result in an embryo with a 2-celled suspensor. In the meantime the endosperm has divided much more rapidly, and contains a large number of nuclei when the embryo is only 4-nucleate. At no time was any sign of a pollen tube seen, either in the micropyle or at the base of the embryo sac, although abundant material of these stages in *A. petaloidea*, *A. neodioica*, *A. canadensis*, and *A. parlinii* was studied.

The second type occurs much less frequently, but some stages of it were found in each of the seven species. Here there is a definite spireme in the young megaspore mother cell (fig. 32), and the spireme contraction or synizesis is present (fig. 33). Diakinesis occurs at the same stage as in the non-parthenogenetic species, but both paired and unpaired chromosomes are present, while the nucleus is very large (fig. 8). At the heterotypic metaphase there are a few chromosomes at the equator (fig. 9), but the majority are scattered irregularly over the spindle. Since there are a number of paired bivalents present, however, this cannot be called a true semiheterotypic division.

The location of the dense layer of the perinuclear zone in figure 9 is interesting. It forms a large loop extending from the micropylar end of the spindle, and is entirely absent from the chalazal end. The inner edge of this loop probably corresponds with the position of the nuclear membrane during diakinesis. The spindle has apparently formed considerably nearer the chalazal end of the nucellus than the original position of the nucleus at diakinesis.

During the early anaphase (fig. 10) the spindle elongates, and reoccupies the micropylar end of the nucellus. Here, and in all the later stages, the major part of the perinuclear zone is at the chalazal end of the nuclear structures. The chromosomes gradually and irregularly move toward the poles, so that they are scattered over the whole length of the spindle. Non-disjunction is common, and paired chromosomes may be seen at both poles. This was the stage most frequently observed of any in the meiotic divisions, and apparently lasts for a considerable period of time. In figure 11 the chromosomes have migrated farther toward the poles, have lost their identity, and are merging together, while a number of them have been ejected into the cytoplasm. The spindle is thinner than ever, and is distinctly bent.

At the heterotypic telophase a large mass of chromatin at the center of the spindle is left out of the two daughter nuclei, and forms a third nucleus (fig. 42). Figure 12 shows a homoeotypic metaphase at which there are three spindles instead of the usual two. This division results in a hexad (fig. 13), although slight irregularities in it frequently cause the presence of small extra nuclei.

The further development of these hexads is problematical. That reduction has taken place is certain, from the distribution of the chromosomes during the heterotypic anaphase; and each cell of the hexad probably has fewer than the normal, haploid number of chromosomes. Figure 49 shows a pentad in which the chalazal cell is developing at the expense of the micropylar ones. This cell has a nucleus distinctly larger than the others, and may have resulted from the fusion of the two daughter nuclei formed by the chalazal spindle of the homoeotypic division. In this case, of course, it would have a number of chromosomes approximating the diploid number for the species.

Figure 50 shows a hexad in which the cell next to the micropylar cell, and that next to the chalazal one, are larger than the other four, and are apparently both starting to develop. Figure 51 shows a very abnormal 4-celled embryo sac, apparently resulting from the situation of figure 50. It is in two parts, the two nuclei at the micropylar end probably having been formed from one cell of a hexad, and the two at the chalazal end from another. The remains of two more of

the hexad cells are seen just below the chalazal end of this "embryo sac." It is doubtful whether such an embryo sac can develop further, and no other similar one was seen at any stage. All the mature embryo sacs are perfectly normal in appearance, and approximate counts in the later divisions showed that the diploid number is most likely present in all where such counts could be made. A few embryo sacs showed obvious signs of degeneration.

In *A. occidentalis*, bagging experiments of the type described in a previous paper (STEBBINS **38**) showed 549 out of 1084 achenes, or 51 per cent, developing in the bagged inflorescences; while in the unbagged material 617 good achenes out of 1106, or 56 per cent, were counted. This difference may be due to a number of causes, such as the more favorable conditions for development in the unbagged heads. The possibility of the fertilization of the egg cells in a few achenes in the unbagged material is slight, as there were no staminate plants of *A. occidentalis* in the vicinity, and the pollen of the staminate plants of the other species had long been shed when the stigmas of *A. occidentalis* first opened. In the other species, in unbagged material, from 50 to 80 per cent of good achenes was found. From the frequency of the second type of development, which will be discussed later, it seems most likely that all of the achenes in which this abnormal reduction division takes place, as well as some in which there is no reduction division, degenerate.

The following material of the various species was studied:

A. occidentalis Greene, no. 536, field, Winchester, Mass.; no. 539, field, Lexington.—In this species the abnormal reduction division is more frequent than in any other, occurring in about 25 per cent of the achenes, 75 per cent developing according to the *A. alpina* type. The diploid chromosome number is about 75–80, although no certain counts could be made.

A. fallax Greene, no. 521, field, Lexington; no. 522, field, Monponsett, Halifax, Mass.—In no. 522, development was entirely of the *A. alpina* type, but in no. 521 various stages of the abnormal reduction division were found in one bud (figs. 32, 34, 35). The chromosome number could not be counted in the pistillate material, but from the staminate plants it is estimated at $2n = 84$.

A. parlinii Fernald, no. 529, meadow, Orange, Mass.; no. 541, hill-

side, Lexington; no. 745, open woods, East Riverdale, Maryland; no 747, field, Ballston, Virginia.—No. 747 differs from the other plants in its smaller, less abundant red glandular hairs, its broader leaves, and its less acuminate, more petaloid involucral bracts, corresponding therefore to var. *arnoglossa* (Greene) Fernald.

In nos. 529 and 541 development is entirely of the *A. alpina* type. In no. 541, the diploid chromosome number, estimated from one of the embryo sac divisions, is about 82. In no. 745, rare evidences of the abnormal reduction division were found, including a tetrad with seven nuclei (fig. 52). In no. 747 the development is mostly of the *A. alpina* type, but one clear case was found of the unreduced megaspore mother cell being replaced by a cell from either the chalazal end of the nucellus or the inner integument (fig. 5). Possible older stages were seen, in which the invading cell, with its dense cytoplasm filled with dark granules, had entirely filled the nucellus, although the remnant of the degenerated archesporial cell could be made out.

A. canadensis Greene, no. 269, dry field, Wilmington, Mass.; nos. 525 and 526, Halifax, Mass.; no. 532, Beverly; and no. 545, Tewksbury.—Development is almost entirely of the *A. alpina* type, the only evidence of a reduction division found being a single old tetrad in no. 526 (fig. 39). However, a single exception to the usual type of metaphase of the first embryo sac division was found in no. 532 (fig. 4). Here the chromosomes are much smaller than in the usual type of metaphase (fig. 3), and are comparatively shorter and thicker. They do not seem to be in pairs, and the division is probably equational; but there are, nevertheless, four chromosomes scattered over the spindle, apparently laggards. The shape of the chromosomes is much the same as in the homoeotypic division of meiosis, and their behavior is apparently the same. No abnormalities preceding this stage were seen, and no prophases of the first embryo sac division were found in no. 532. The metaphase, in appearance and distribution of the chromosomes, resembles the only one seen and illustrated by JUEL (**21**) in the first embryo sac division of *A. alpina*. Here he remarks on the presence of a few chromosomes not at the equator of the spindle, and suggests that they have been displaced by the knife. In the case of no. 532, however, the cell plate was not touched in sectioning.

A. petaloidea Fernald, no. 538, field, Arlington Heights; no. 527, Bryantville, Mass.—The latter plant corresponds to var. *noveboracensis* Fernald. In no. 538, very few of the younger stages were observed, and these all showed the *A. alpina* type of development. In no. 527, the abnormal reduction division occurs in about 20 per cent of the achenes (figs. 40–43). In the homoeotypic metaphase in figure 43, about 36 chromosomes can be counted on one spindle and about 40 on the other. No other chromosome counts could be obtained in *A. petaloidea*, so that the diploid chromosome number can be estimated at 75–80.

A. neodioica Greene, no. 268, clearing, Wilmington, Mass.; no. 523, field, Monponsett; no. 533, clearing, Beverly Farms, Mass.—In nos. 268 and 533, only the *A. alpina* type of development was found; but in no. 523 the abnormal reduction division occurs in about 10–15 per cent of the achenes (figs. 44–47). Such chromosome counts as could be made from these stages fixed the diploid number as near 52.

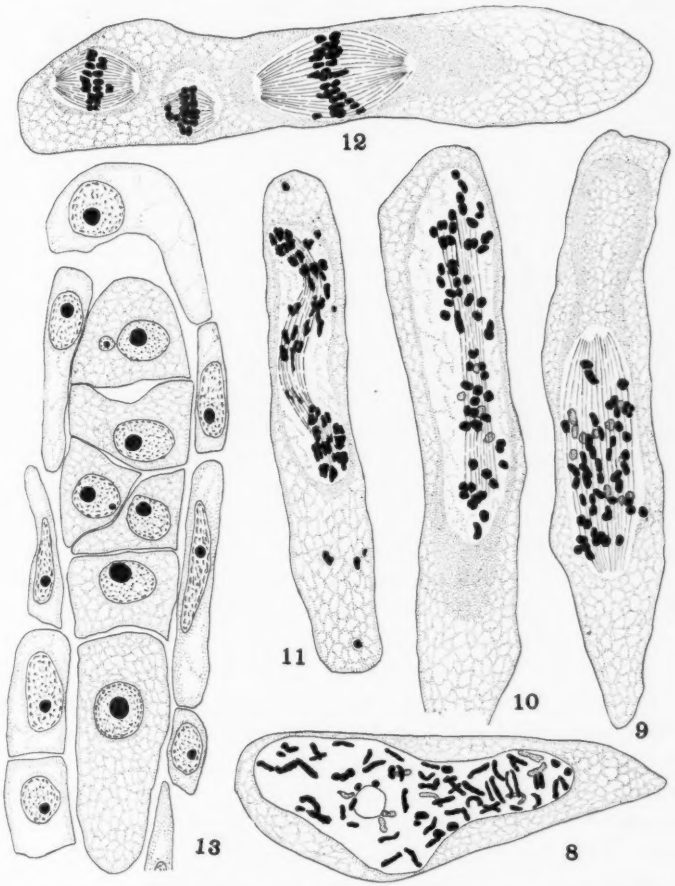
A. neodioica Greene var. *attenuata* Fernald, no. 266, open woods, Dedham; no. 534, clearing, Beverly Farms; no. 758, Canton Junction.—In all of these the development is entirely of the *A. alpina* type. Chromosome counts could not be obtained.

A. brainerdii Fernald, no. 270, open pine woods, Tewksbury, Mass.—The development is mostly of the *A. alpina* type, but a single heterotypic anaphase was found (fig. 48). Here the chromosomes are characteristically scattered, and the number counted is 42, which is about the diploid number for *A. brainerdii*, since in this figure it is certain that no chromosomes have been cut away. No other counts could be obtained.

Discussion

TYPES OF APOMIXIS.—The terminology to be used for the various types of apomixis has long been subject to a difference of opinion, there being three main classifications of the various phenomena, given by STRASBURGER (**39**), ERNST (**14**), and WINKLER (**43, 45**). Since WINKLER'S purely morphological classification is the most definite, and has been generally adopted in recent years (ROSENBERG **35**), it is the basis for the terminology in this study. According to this terminology the development in the seven species here studied is

diploid parthenogenesis, and where it is completed is, as already noted, of the *Antennaria* type (ROSENBERG **35**). This is the most



FIGS. 8–13.—Fig. 8, *A. occidentalis*, abnormal reduction division, diakinesis; fig. 9, heterotypic metaphase; fig. 10, early anaphase; fig. 11, late anaphase; fig. 12, homoeotypic metaphase; fig. 13, hexad. ×1800.

common type of diploid parthenogenesis, and a list of the large number of species possessing it is given by ROSENBERG.

Apospory was noted in *A. neglecta*×*plantaginifolia* (STEBBINS 38), but was not found in any of the species here studied. However, a transition between true apospory and the *Antennaria* type of parthenogenesis is exhibited by *A. parlinii* no. 747, where the original archesporial cell is replaced by an integumentary one, which develops directly into the embryo sac. This replacement of the original archesporial cell by a purely somatic one is found also in *Alchemilla* (MURBECK **25,** STRASBURGER **39**) and *Oxyria digyna* (EDMAN **13**).

Since apomixis generally involves an elimination of the reduction division, or of its resultant haploid cells, from the life cycle of the organism, a comparison of the reduction division and the chromosome constituency of apomictic forms with that in other types of organisms where this division is eliminated or disturbed will clearly throw light on the origin of apomixis.

MEIOTIC IRREGULARITIES.—Irregularities in the reduction division, with their consequent microspore and megaspore degeneration and sterility, are well known to occur regularly in apomictic forms. JUEL (**21**) noted the complete pollen sterility of *Antennaria alpina* in herbarium specimens, and for that reason supposed it to be of hybrid origin. ROSENBERG (**31, 33**) noted abnormalities in the various apomictic species of *Hieracium;* while HOLMGREN (**19**) in *Erigeron,* OSAWA (**27**) in *Taraxacum,* and WOODWORTH (**47, 48**) in *Alnus* noted the irregularity of the apomictic as contrasted with the regularity of the reduction division in the sexually reproducing forms.

The irregularities in meiosis may be abnormalities, either in behavior and distribution of the chromosomes or in the condition and activity of the remainder of the nucleus and the cytoplasm. Chromosome irregularities, consisting chiefly of the failure of the chromosomes to pair and their consequent lagging and extrusion into the cytoplasm, are characteristic of interspecific hybrids, as in *Drosera* (ROSENBERG **32**), *Crepis* (COLLINS and MANN **9**), *Viola* (CLAUSEN **7**), and many other genera. The types of irregular division in the pollen mother cells have been well classified by ROSENBERG (**34**) according to the degree of pairing, as shown by meiosis in the pollen mother cells of species of *Hieracium.* In *Antennaria fallax* and *A. parlinii* the division is of the least irregular, or *H. boreale* type. The dyads in *A. parlinii* are due to a chance bridging of the space

between the daughter chromosome groups in the late heterotypic anaphase by a group of univalents, as described by MATSUDA (**23**) in *Petunia violacea*, rather than to a true semiheterotypic division.

In the pollen mother cells of *Antennaria canadensis* the *H. laevigatum* type, or semiheterotypic division, occurs. This type, described by ROSENBERG (**33**) in the apomictic *Hieracium* species, has been found in a number of forms, including many known hybrids and apomictic forms.

The abnormal reduction divisions in the megasporocytes of the parthenogenetic species of *Antennaria* are intermediate between the *boreale* and the *laevigatum* type. Although few or none of the chromosomes form bivalent pairs, and the univalents are scattered throughout the length of the spindle, the "restitution nucleus" never forms, probably due to the length and slenderness of the spindle. A heterotypic division similar to this was described by CHIARUGI (**5**) in *Artemisia nitida*. This division, however, was not followed by a homoeotypic one; and the homoeotypic division with three spindles, found in the megasporocytes of the parthenogenetic species of *Antennaria*, has not apparently been described in the megasporocytes of any other plants. It does, however, resemble the phenomenon described by SEILER (**37**) in the ovogenesis in the lepidopteran genera *Lymantria*, *Phragmatobia*, and *Orgyria*, and interpreted as chromatin diminution. In these cases, however, a metaphase cell plate is formed, and the chromosomes break and leave some of their chromatin at the equator. This, together with univalents, forms a nucleus during interkinesis.

Extra-chromosomal irregularities, consisting of abnormalities of cytoplasmic behavior, occur in the parthenogenetic species of *Antennaria*. The sporocytes in these forms often have a thin, evacuolate cytoplasm, which sometimes behaves anomalously. This is shown most clearly by the changes in position of the spindle and perinuclear zone during the heterotypic division in the parthenogenetic forms. The perinuclear zone was first recognized by BYXBEE (**3**) in *Lavatera*, and by CANNON (**4**) in a hybrid cotton, and has since been observed by many workers. In the megasporocyte meiosis of the parthenogenetic species of *Antennaria*, its usual greater abundance at the micropylar end of the spindle during the heterotypic

metaphase, and at the chalazal end during the later stages, accompanied by a corresponding change in position of the spindles in the cytoplasm, indicates considerable shifting of the nuclear and cytoplasmic material back and forth in the cell. This was not observed at all in the normal, sexual species (STEBBINS **38**), and is evidence of considerable physical disturbance in the cytoplasm.

Another cytoplasmic phenomenon, found more frequently in the parthenogenetic species of *Antennaria* than in the normal ones, and generally in hybrids and forms with irregular meiosis (DIGBY **12**), (HICKS **18**), (CHURCH **6**), is that of cytomixis, first observed by GATES (**17**) in *Oenothera gigas*. It is most abundant in the prophase, but CHURCH found it in the heterotypic anaphase of *Panicum sphaerocarpon*, and WOODWORTH (**46**) in the homoeotypic metaphase of *Betula papyrifera* var. *cordifolia*. Its occurrence as late as the homoeotypic telophase of *A. fallax* is unusual. It has been attributed by ROSENBERG (**49**) and others to faulty fixation, and by WOODWORTH (**49**) to pressure against the pollen mother cells during the collection of the material; but its greater abundance in cells with irregular division, those subject to chloralization (SAKAMURA **36**) and abnormal temperatures (INARIYAMA **20**), indicates that, whether an artifact or not, it reflects in these cases an abnormal state of the cytoplasm, which may often be due to a hybrid condition.

To abnormal conditions in the cells as a whole must be attributed the irregular development of the megaspores formed by the irregular divisions in the parthenogenetic species of *Antennaria*. Cases of the development into an embryo sac of other than the usual chalazal megaspore are found in normal species (AFZELIUS **1**), but the development at an approximately equal rate of two megaspores to form different parts of one embryo sac, as in *A. occidentalis*, is distinctly an abnormal condition. A similar case is in the hybrid *Senecio nebrodensis*×*viscosus* (AFZELIUS), although here one of the two cells completes development by itself into an embryo sac. Apparently the balance in the physiological condition of the megaspores has been so upset by the irregular meiosis that no one of them has the same faculty to develop as has the chalazal megaspore in normal plants.

POLYPLOIDY.—That apomictic forms generally have higher chromosome numbers than their most nearly related normal species has

been recognized by all students of apomixis, such as STRASBURGER (39), ROSENBERG (35), WINKLER (43), and ERNST (14). In *Antennaria* the parthenogenetic species all have higher chromosome numbers than their normal relatives, the diploid numbers varying from 42 to 84, while that in all of the normal species studied is 28.

Polyploid forms are often larger and more robust than their nearest related diploid ones, as is shown by such tetraploid races as *Oenothera gigas* (GATES 16), and *gigas* forms of *Solanum* (WINKLER 44). The polyploid species of *Antennaria* here studied are in general taller and more robust than the sexually reproducing species.

A physiological characteristic of the polyploid species of *Antennaria*, as well as those of *Crepis* (NAWASCHIN 26), and probably other genera, is their slower rate of development. These species of *Antennaria* in the vicinity of Boston bloom about three weeks later than their relatives with a smaller chromosome number.

Polyploid forms are of two main types as regards their nature and origin. Autopolyploids, which have arisen in genetical cultures of several types of plants, such as *Oenothera* (GATES 16), *Solanum* (WINKLER 44), and *Datura* (BELLING and BLAKESLEE 2), contain the chromosome sets of their diploid progenitors duplicated, and do not differ morphologically from them except in their larger size throughout. Allopolyploids have arisen from interspecific hybridization. The first case reported of such a polyploid hybrid is that of *Primula kewensis* (DIGBY 11), and there are many other cases now known, such as those in *Nicotiana* (CLAUSEN and GOODSPEED 8), *Viola* (CLAUSEN 7), *Brassica*×*Raphanus* (KARPECHENKO 22), and *Galeopsis* (MÜNTZING 24). These forms are usually intermediate in their morphological characteristics between their two parents; or they may, as in the case of *Galeopsis tetrahit* (MÜNTZING), have characters distinct from either parent. Allopolyploids, if the chromosomes of their parents are somewhat homologous, may have one or more quadrivalents, with secondary pairing at the heterotypic metaphase, as in *Primula kewensis* (DIGBY 11) and *Rubus rusticanus* var. *inermis*×*R. thyrsiger* (CRANE and DARLINGTON 10); but more frequently such multivalent groups are completely absent.

Whether the parthenogenetic species of *Antennaria* are to be interpreted as auto- or allopolyploids is a problem. *A. fallax* and *A.*

occidentalis resemble, except for their increased size, races of *A. plantaginifolia;* but since they are hexaploids or nearly so with reference to this species, their origin is undoubtedly more complex than that of *Oenothera gigas* and similar forms. Quadrivalent and sexivalent associations are not so general in *A. fallax* as would be expected if the species were a true autopolyploid. Such associations are more frequent in *A. parlinii*, but this species differs from all other known species in other distinctive characteristics besides size. The other parthenogenetic species do not resemble closely any single normal sexual species, and are therefore most likely allopolyploids. Since this type of polyploid has by definition originated through hybridization, and since the parthenogenetic species of *Antennaria* show the characteristics of hybrids and of species of hybrid origin in their reduction divisions, the most plausible theory for their origin is that of ERNST (**14**) and WINGE (**42**) for parthenogenetic forms in general, that they have originated through interspecific hybridization.

ORIGIN OF PARTHENOGENETIC SPECIES.—Although the cytological evidence points to a hybrid origin for the parthenogenetic species of *Antennaria*, these species undoubtedly originated many thousands of years ago, and any attempt to discover their parentage is fraught with difficulty.

A. fallax differs from *A. plantaginifolia* only in its larger size throughout, and higher chromosome number. It may have evolved from *A. plantaginifolia* simply by a trebling of the chromosome number, but that hypothesis is improbable, as already noted. More likely *A. fallax* has resulted from a back cross from some hybrid of *A. plantaginifolia* with the parent species, and has thus acquired a preponderance of *A. plantaginifolia* chromosomes. *A. occidentalis* is likewise closely related to *A. plantaginifolia;* but in its obovate, rounded leaves, and its stolons, often longer and more procumbent than in *A. plantaginifolia*, it shows a transition toward *A. neglecta*, or possibly *A. solitaria*, and could have arisen from a back cross from a hybrid between *A. plantaginifolia* and any one of these species to *A. plantaginifolia*.

A. petaloidea, as pointed out by FERNALD (**15**), resembles *A. neglecta*, differing in its larger, stouter habit and its corymbose inflorescence. The increased size is a result of the trebling of the chro-

mosome number, while the corymbose inflorescence is a character of *A. plantaginifolia*. Furthermore, some plants of *A. neglecta* × *plantaginifolia* in the writer's collection (nos. 495, 514, and 535) resemble *A. petaloidea* except for their reduced size. *A. petaloidea* may therefore be explained as a polyploid, parthenogenetic form which arose from a cross between *A. neglecta* and *A. plantaginifolia*, in the manner of *Primula kewensis* and *Galeopsis tetrahit*, and has spread northward from its place of origin beyond the range of either of its parents.

The other species are more obscure in their affinities. If they are of hybrid origin, one or both of their ultimate parents must now be extinct or absent from the regions that they formerly occupied. Some of the multitudinous varieties and forms must have arisen in one of the following ways.

Hybridization between partially apomictic forms may account for such forms as the series of intermediate races which connect *A. parlinii* with *A. fallax* and *A. occidentalis*. In New England, where staminate plants of *A. parlinii* are almost unknown and those of *A. fallax* are rare, these intermediates are rare or not known; while in the vicinity of Washington, D.C., in Indiana, and the Mississippi Valley, where staminate plants, particularly of *A. parlinii*, are much more abundant, the intermediate races are likewise very abundant and puzzling to the systematist. It seems likely that here hybridization has taken place between *A. parlinii* and its relatives, either through the pollination of the ovules in the "aberrant male" flowers, where the reduction division is not very irregular and functional gametes must be frequently formed, or through fertilization of rare functional gametes formed by an occasional successful reduction division in the pistillate flowers. Such hybridization was produced in the partially apomictic *Hieracium* species by OSTENFELD (**28, 29**), and is considered by him an important cause of the multiplication of closely related apomictic forms in that genus.

Many varieties and species of a local, restricted range, such as *A. rupicola* Fernald and *A. petaloidea* vars. *noveboracensis* and *subcorymbosa*, have very likely arisen from more widely distributed relatives by the process described in the apomictic species of *Hieracium* by OSTENFELD (**29, 30**) as apogamic mutation. A possible mecha-

nism for this process is seen in the abnormal first embryo sac division of *A. canadensis* (fig. 4). If the lagging chromosomes in this division failed to reach the proper poles, an egg cell with one or two more or less than the diploid chromosome number would be formed, which, if capable of parthenogenetic development, would produce an offspring with a slightly different chromosome number and hence with different characteristics. Unfortunately, the large numbers of chromosomes in all these forms made it impossible to detect with any certainty slight differences in number between them.

Thus it is seen that in some of the parthenogenetic species of *Antennaria*, both the cytological and morphological evidence points to their hybrid origin; while in the others, the theory that they arose through hybridization a long time ago and have since reproduced themselves by parthenogenesis, while one or both of their parent species have become extinct, at least within their range, is a distinctly probable one. Hence the theory of ERNST and WINGE may be said with some certainty to apply to *Antennaria* as well as to other genera where apomixis is prevalent.

Summary

1. In *Antennaria fallax* the haploid chromosome number is 42, and meiosis in the pollen mother cells is irregular.

2. In the megaspore mother cells of male intersex flowers of *A. fallax*, meiosis is likewise irregular.

3. In *A. parlinii* the haploid chromosome number is also 42, and meiosis in the pollen mother cells is irregular, sometimes failing of completion.

4. In *A. canadensis* meiosis is very irregular, and is frequently a semiheterotypic division, resulting in the formation of dyads of microspores.

5. In the pistillate flowers of all the seven species studied, the embryo sac originates most often directly from an unreduced megaspore mother cell. The egg cell of such an embryo develops directly, without fertilization, into an embryo.

6. In all seven species, the megaspore mother cell occasionally undergoes a very abnormal reduction division. This division usually produces three nuclei instead of two, and the homoeotypic division following it forms a hexad or other polysporic group.

7. Evidence makes it likely that the products of this division generally degenerate.

8. In *A. parlinii* a peculiar type of apospory was found.

9. In *A. canadensis* an abnormal first embryo sac division was found.

10. The approximate diploid chromosome numbers are: *A. fallax* 84; *A. occidentalis* 75–85; *A. parlinii* 84; *A. canadensis* 83–86; *A. petaloidea* 75–80; *A. neodioica* ca. 52; *A. brainerdii* 42.

11. The development in the seven species is diploid parthenogenesis of the *Antennaria alpina* type.

12. In their irregular meiosis, the parthenogenetic species of *Antennaria* resemble other apomictic forms, and known hybrids.

13. Various extra-chromosomal irregularities of meiosis are discussed.

14. The species are all allopolyploids resembling those which have originated through interspecific or intergeneric crossing.

15. Some of the parthenogenetic species are morphologically intermediate between the normal species.

16. The parthenogenetic species have most likely originated from crosses between existing or extinct normal, sexually reproducing species.

The writer wishes to acknowledge with thanks the assistance of Professor E. C. JEFFREY, who suggested the problem and whose advice and technical direction have been invaluable throughout the work. Thanks are also due to Dr. S. F. BLAKE for assistance in obtaining material of the species growing in the vicinity of Washington, D.C.

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EXPLANATION OF PLATES II, III

PLATE II

All figures, ×2400.

FIG. 14.—*Antennaria fallax*, microsporogenesis, heterotypic metaphase.

FIG. 15.—Same, polar view showing 42 bivalent chromosomes and 4 univalents (in stipple).

FIG. 16.—Heterotypic anaphase showing laggards and cytoplasmic connections with a neighboring cell.

FIG. 17.—Homoeotypic metaphase.

FIG. 18.—Homoeotypic telophase showing cytomixis.

FIG. 19.—*A. parlinii*, microsporogenesis, heterotypic metaphase.

FIG. 20.—Same, showing 37 bivalent and 10 univalent chromosomes.

FIG. 21.—"Restitution nucleus" at interkinesis of microsporogenesis.

FIG. 22.—Polysporic heptad.

FIG. 23.—"Dyad" with two extra microcytes.

FIG. 24.—*A. canadensis*, early semiheterotypic anaphase of microsporogenesis.

FIG. 25.—Late semiheterotypic anaphase, showing bent spindle.

FIG. 26.—Restitution nucleus.

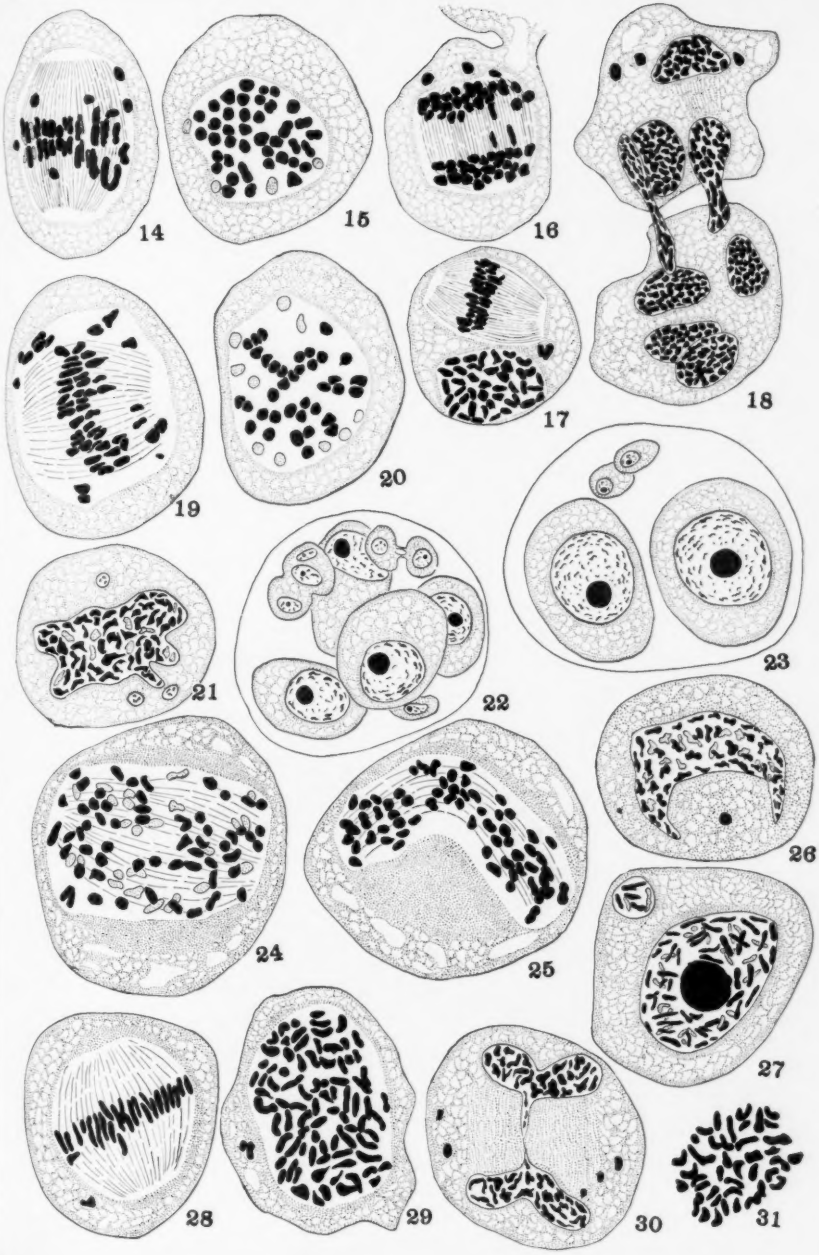
FIG. 27.—Same, later stage showing splitting of chromosomes; extra micronucleus present.

FIG. 28.—"Homoeotypic" division of restitution nucleus, metaphase.

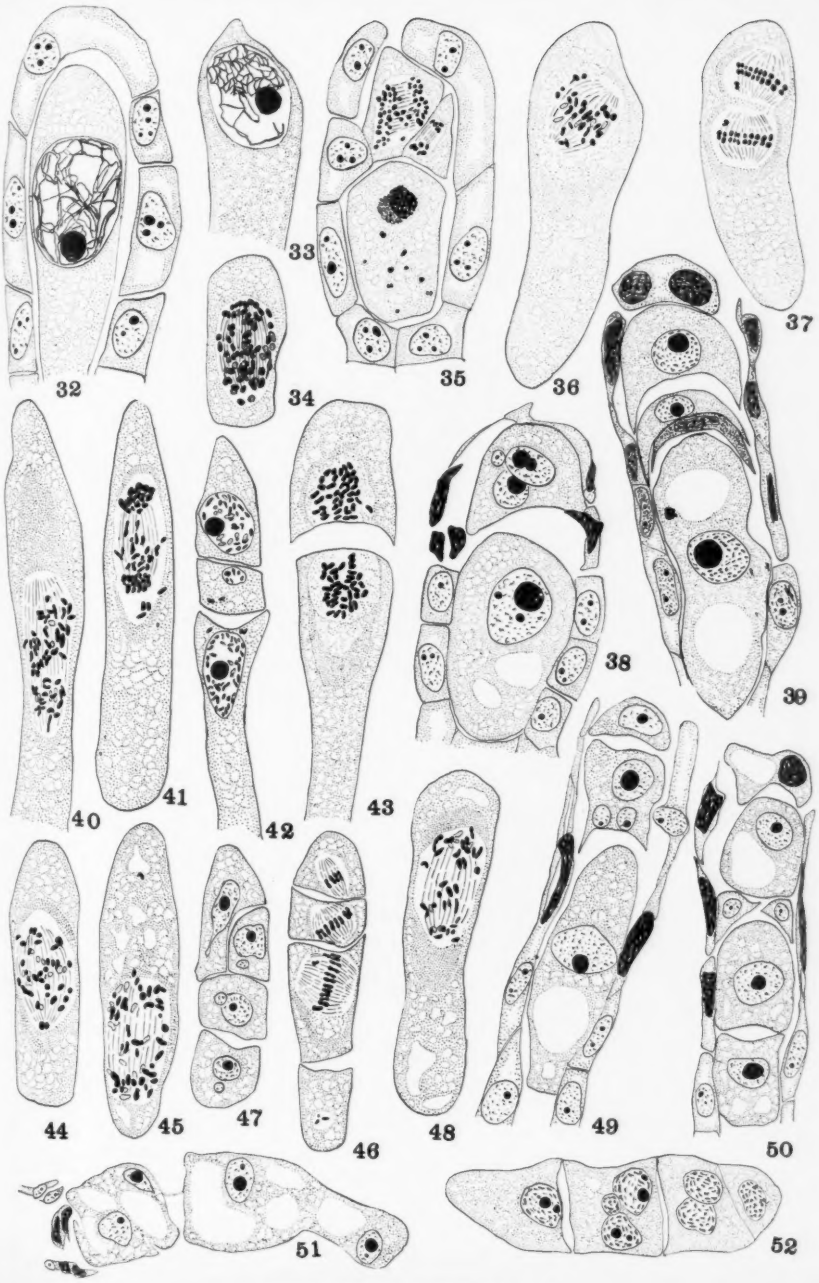
FIG. 29.—Same, polar view.

FIG. 30.—Telophase of same.

FIG. 31.—Cell plate, polar view, of regular homoeotypic metaphase.



STEBBINS on ANTENNARIA



STEBBINS on ANTENNARIA

PLATE III

Figure 51, ×600; all others ×1200.

FIG. 32.—*Antennaria fallax*, prophase of abnormal reduction division showing spireme.

FIG. 33.—*A. parlinii*, same showing synizesis.

FIG. 34.—*A. fallax*, abnormal reduction division, heterotypic metaphase.

FIG. 35.—Homoeotypic anaphase of same.

FIG. 36.—Megasporogenesis in "aberrant male" flowers, heterotypic metaphase.

FIG. 37.—Homoeotypic metaphase of same.

FIG. 38.—Tetrad in "aberrant male" flowers.

FIG. 39.—*A. canadensis*, tetrad of megaspores.

FIG. 40.—*A. petaloidea*, heterotypic metaphase.

FIG. 41.—Heterotypic anaphase.

FIG. 42.—Interkinesis, showing three cells.

FIG. 43.—Homoeotypic metaphase.

FIG. 44.—*A. neodioica*, heterotypic metaphase.

FIG. 45.—Heterotypic anaphase.

FIG. 46.—Homoeotypic metaphase showing four cells and three spindles.

FIG. 47.—Tetrad with microcytes.

FIG. 48.—*A. brainerdii*, heterotypic early anaphase.

FIG. 49.—*A. occidentalis*, pentad.

FIG. 50.—Old hexad, two cells developing.

FIG. 51.—Abnormal embryo sac.

FIG. 52.—*A. parlinii*, tetrad.

METHODS FOR CONTROLLING THE ENVIRONMENT OF GREENHOUSE PLANTS[1]

P. W. Wilson and C. E. Georgi

(with three figures)

Introduction

In all physiological experiments on plants made in the greenhouse, it is desirable to control, in so far as is possible, the environment under which the plants are grown. Although this problem doubtless arises frequently among workers in plant physiology, agronomy, and plant chemistry, there are surprisingly few contributions concerned with simple and comparatively inexpensive means of controlling such an environment.

In most physiological and chemical studies on plants, there has been a tendency either to ignore entirely the possible effects of changes in light, humidity, temperature, etc., or to control these within very narrow limits by means of elaborate and often expensive equipment. The second method is certainly to be preferred, but it is not always available to the majority of investigators in plant physiology. The technique developed at the Boyce Thompson Institute (**2, 3**) for maintaining constant conditions for the plants is desirable but not practicable for most investigators, both from the financial and technical points of view. The less expensive constant-condition chambers of the type described by Davis and Hoagland (**4**), Tottingham (**19**), and Steinberg (**17**) have the objection that the space controlled is limited and therefore cannot be used for extensive experiments.

It would seem highly desirable that a technique be developed suitable for large-scale experiments to be used in greenhouse studies. Such a technique would use as a basis the results already obtained in the more elaborate arrangements referred to, but would seek to

[1] Herman Frasch Foundation in Agricultural Chemistry, Paper no. 46. Contribution from the departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

apply them to actual working conditions in the greenhouse. As examples of the possibilities of this application to the control of rooms capable of large-scale experiments may be cited the constant-condition rooms described by SANDE BAKHUYZEN (**14**) or HARVEY (**7**). While the exact control obtained in the constant-condition chambers, or even in the larger rooms, could not be expected in greenhouse work, at least a partial control over the environment might be achieved. It must be remembered also that any efforts to regulate light, atmosphere, humidity, and temperature lead to an appreciation of the influence of these factors upon the results, and a more nearly correct interpretation of the data is therefore possible.

Experimentation

GREENHOUSE.—The greenhouse used in this work is a unit in a series that face north; at the southern end it is joined to the headhouse, which partially shades this end. The unit is 36 feet long and 22 feet wide. Each side is lined with a bench 3.5 feet wide and 3 feet in height. In the center of the house and separated by a cement walk 3 feet wide, are two benches that extend from the north end for 15 feet and are 4 feet wide and 4 feet in height. The remainder of the length of the greenhouse in the center is occupied by benches of the same dimensions, except that the height is only 3 feet. The benches are so constructed that there is a minimum of obstruction under them, thus allowing free circulation of air; the steam pipes used to heat the house in the winter are located under the side benches. On the top of each bench is a 2×4 railing; this forms a shallow bed which is lined with tar paper and filled with wet sand in which are placed the plant cultures. Sand is also placed under the beds and is kept moist by frequent sprinkling, as are the concrete walks that surround the beds. Solid $CuSO_4 \cdot 5H_2O$ is kept on the wet sand beds and on top and under the benches, in order to prevent the growth of algae. At the extreme south end of the house the side benches are not covered with sand, and a space 5 feet in length is reserved for laboratory work. Here the plant cultures are put up and certain routine chemical analyses made.

GAS MIXTURES.—In many physiological problems it is necessary to regulate the partial pressures of the gases supplied the plants,

especially the pCO_2 and pO_2. The method to be described was developed for study of small leguminous plants, but it could be extended to larger species by modification of the container for the plant cultures. Since a majority of studies concerned with the gase-

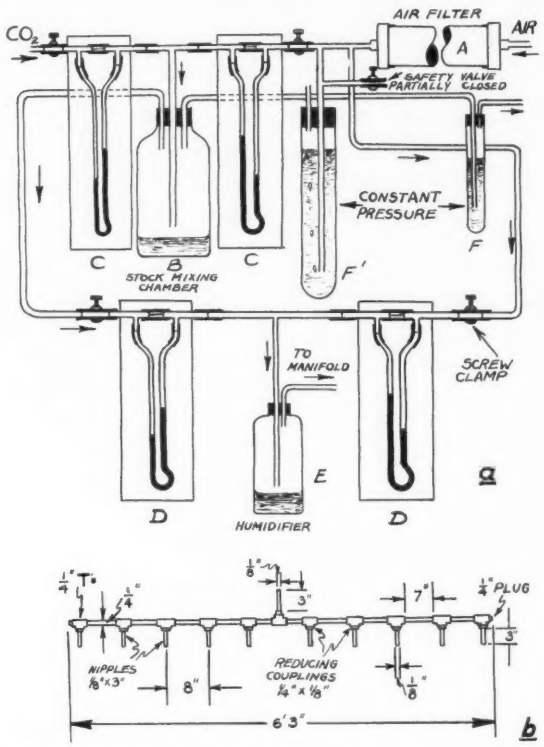


FIG. 1.—Diagram of apparatus for regulation of pCO_2 in atmosphere

ous environment of the plant deal with the effect of pCO_2, a detailed description will be given of the method used to regulate the partial pressure of this gas.

A diagram of the system used to supply atmospheres of various CO_2 content is shown in figure 1. Air from a compressed air-line is passed over concentrated H_2SO_4, then through a 2.5-inch pipe (*A*)

32 inches in length which has been filled with cotton and sterilized. The air current is then divided and a measured quantity combined in the stock mixing chamber (*B*) with a known volume of CO_2 obtained from a commercial cylinder. The volume of each component of the resulting mixture is measured by means of the flow meters (*C*) which have been standardized to deliver a known volume of gas per unit of time for any given difference in pressure registered. In the mixing chamber is kept a saturated solution of NaCl which aids in regulating the humidity of the gas mixture. The air plus CO_2 is then mixed with ordinary air, as indicated in figure 1 *a* (flowmeters *D*) to make an atmosphere of the desired CO_2 content. The mixing is done in the humidifier (*E*), which contains a saturated NaCl solution, and then passes to a manifold for distribution among the plant cultures.

For example, an air-CO_2 mixture which contains 0.8 per cent CO_2 is made by passing air into *B* at the rate of 5000 cc. per minute and CO_2 at the rate of 40 cc. per minute. An atmosphere, which has a CO_2 content of 0.2 per cent, can now be made by mixing in *E* 620 cc. of air (0.03 per cent CO_2) and 180 cc. of the 0.8 per cent CO_2 mixture. The 0.2 per cent mixture is thus delivered to the manifold at the rate of 800 cc. per minute; it is obvious that any other rate could be supplied by modification of the volumes of the two components used. In order to simplify figure 1, only one set of flowmeters for supplying a manifold is indicated, but actually five or six can be taken care of by one stock mixing chamber. More stock mixture (0.8 per cent CO_2) is made than is needed for the cultures, and the excess is carried from the greenhouse through the constant pressure device (*F*).

For distributing the gas mixtures to the manifolds 0.25-inch copper tubing is very satisfactory, as it is inexpensive, flexible, and durable; glass tubing can be used but it is not so convenient as the copper tubing. A heavy-walled red rubber tubing is used for the necessary rubber connections; a high grade will last at least six months in greenhouse use. For delivering the gas mixture to the plants, a manifold of the type detailed in figure 1 *b* can readily be made. The construction of the flowmeter is shown in detail in figure 2 *a*. The glass-stoppered drying tube is used to regulate the flow of gas and to collect excess moisture in the gas; this tube is not absolutely necessary since ordinary screw clamps are satisfactory for regulation, as indi-

cated in figure 1*a*. The capillary tubing varies in size from 0.1 to 2 mm., depending upon the rate of flow desired. Since the proper size will also depend upon the pressure at which the gases are delivered (height of water in F and F_1), the correct size for a given rate

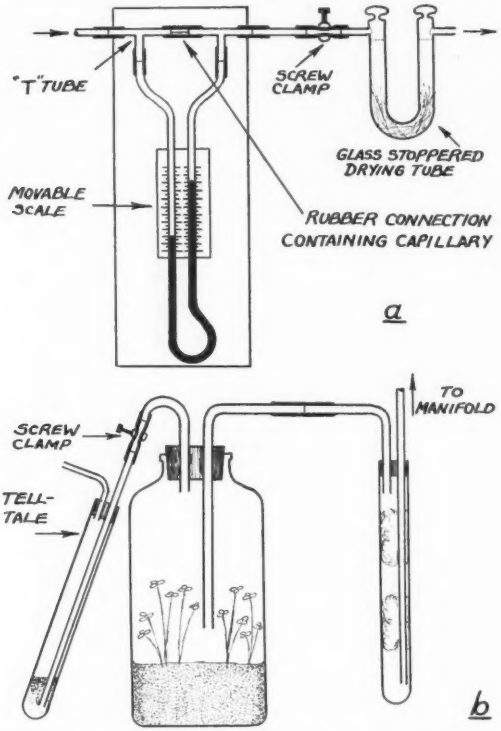


FIG. 2.—Details of flowmeter and culture bottle, approximately one-third actual size.

of flow is best determined by trial. The liquid used for an indicator in the flowmeters is a matter of choice, but one with a low vapor pressure at ordinary temperatures is desirable; n-butyl phthalate colored with methyl red dye is very satisfactory.

To standardize a flowmeter, the gas mixture which it is to measure is passed through the capillary tube under a pressure sufficient

to maintain a given difference in the levels of the liquid in the arms of the U-tube; the time required for a known volume of the gas to pass through is determined and is calculated to cc. per minute. This is repeated for several readings of the flowmeter, and from these data a calibration curve is constructed. Since for small differences in pressure the rate of flow through the capillary is a straight line function of the difference in pressure, three determinations usually will suffice for a calibration.

The plants are grown in 64-oz. bottles of clear flint glass in which a substrate of Crone's agar or sand plus Crone's solution is used. The details for planting under bacteriologically controlled conditions have been described in a previous publication (**8**). After planting, the cotton plug is replaced by the apparatus shown with the bottle in figure 2 *b;* this unit is sterilized separately in wrapping paper just before use. The gas from the manifold is filtered through sterile cotton before it passes over the plant. The rate of flow through each bottle on a given manifold is kept fairly uniform by regulation with a screw clamp on the tell-tale; the latter contains $HgCl_2$ solution (1:1000). The method just described is applicable only to experiments in which plants are grown in a closed system; if this is not desirable, a modification can be used. The plants are grown on sand in 64-oz. bottles which are left open to the air; the gas mixture from the manifold passes through a tell-tale, and is then led by a bent glass tube to the surface of the sand where it must diffuse among the plants before escaping into the greenhouse. The use of a bottle prevents air currents from mixing the added CO_2 with the surrounding air as soon as it is delivered to the plants. With clover and alfalfa this system was very satisfactory, as the plants grew out of the tops of the bottles to a large size and were in every way comparable with plants grown in open sand pots.

ILLUMINATION.—If considerable work is done during the winter months, the question of sufficient light for plants is a most important problem. The lack of sunlight in this latitude becomes very acute from November until March, as is indicated by the meteorological data for this period. The average daily sunlight for these months at Madison, Wisconsin, is: November 3.9 hours, December 3.3 hours, January 4.0 hours, and February 5.2 hours. Also the effect of the

lack of sunshine is even greater than these data indicate, since the average values are given; a perusal of the daily reports for these months shows that there are many days in which no sunshine appears. This leads to an interrupted, erratic growth of plants, unless the deficiency in light is compensated in some way. The use of artificial lights to supplement or replace sunlight in plant experiments has been investigated by many workers and the results have been most encouraging. SHIRLEY (16) has recently reviewed the various sources available, with comments on their efficacy; representative papers concerned with the effect on plant growth of artificial illumination are those of HARVEY (7), DAVIS and HOAGLAND (4), TJEBBES and UPHOF (18), SHIRLEY (15), and HARDER, KEPPLER, and REUSS (6).

The question of the type of lamp to use as a source of artificial light is a difficult one; 100 to 1500-watt lights have been used with varying success, and the decision usually rests upon considerations other than mere intensity and quality.

A study of the papers dealing with growth of plants under artificial lights indicates that satisfactory results are obtained with an intensity from 400 to 1000 foot-candles; however, in most cases the electric lamps were used as the sole source of radiant energy, so that somewhat lower intensities would probably be satisfactory if the lights are employed to supplement and not to replace sunlight. Table I, constructed from data given by LUCKIESH (12), gives the light characteristics of Mazda lamps of various watt ratings compared with sunlight. The intensities were calculated on the assumption that reflecting devices were available which would allow 75 per cent of the total radiation to be spread uniformly over a horizontal space 4 square feet in area. These data show that as the watt rating increases, the lamp becomes more efficient (lumens per watt) and the distribution of energy more favorable for plant growth; that is, it is displaced toward the lower wave lengths.

In spite of these characteristics, the use of high wattage lamps is not always feasible in greenhouses because of the expense and heating effects. The heat production (infra-red radiation) is given in the last two columns of table I; the last column gives the rate at which heat is supplied to an area of 4 square feet on the assumption that the infra-red radiation is reflected and transmitted to the same de-

gree as are the visible wave lengths. It is apparent from this table that unless means are available to remove the infra-red radiation (for example, water screens), it will be necessary to keep the high rating lamps farther from the plants and to spread the total energy flux over a large area. If this is done, the visible illumination is likewise decreased in intensity so that there is usually a limit in ordinary greenhouse practice to the intensity of light that can be supplied the plants from electric lamps. For example, if the radiation from a

TABLE I

LIGHT CHARACTERISTICS OF MAZDA LAMPS AND SUNLIGHT

SOURCE OF LIGHT	COLOR TEMPERATURE (° K)	LUMINOUS EFFICIENCY (LUMENS PER WATT)	INTENSITY* (FT.-CANDLES)	PERCENTAGE DISTRIBUTION OF ENERGY†				HEAT PRODUCTION	
				< 3000 A°	3000–4000 A°	4000–7600 A°	> 7600 A°	PER FT.-CANDLE (CAL./MIN./CM².)	TOTAL ON 4 SQ. FT (CAL./MIN.)
I. MAZDA LAMPS									
50 watt . . .	2670	10.0	94	0.002	0.85	9.2	90.7	1.40×10^{-3}	49
100 " . . .	2740	12.9	242	0.003	0.11	10.1	89.8	1.06 "	975
200 " . . .	2810	15.2	566	0.004	0.14	11.1	88.7	0.90 "	1900
500 " . . .	2920	18.1	1700	0.008	0.20	12.7	87.1	0.74 "	4670
II. SUNLIGHT‡									
June	5000–6000	. . . . .	8540	0.022§	3.9§	43.7	52.4	0.094 "	2970
February		. . . . .	4840	0.006	2.8	42.1	55.1	0.154 "	2820
December		. . . . .	2415	0.0005	1.6	39.1	59.3	0.281 "	2520

* Values for the Mazda lamps were calculated on the assumption that reflection is 75 per cent efficient and that the total radiation is uniformly spread on a horizontal surface, 4 sq. ft. in area.

† Per cent of total radiation.

‡ Data are for sunlight in Cleveland, Ohio.

§ Values for the sun's radiation are for 2900–3100 A° and 3100–4000 A° respectively.

500-watt lamp is distributed over an area such that the infra-red radiation supplied per unit area per unit of time is equal to that of a 200-watt lamp, the visible light supplied will be about 20 per cent higher in the case of the 500-watt lamp. This calculation, however, is based on the assumption that the reflection and transmission of the various wave lengths are the same, and since this is not true it must be corrected. The longer wave lengths are more readily transmitted through air and reflected from surfaces; therefore the value is too high. In order to accomplish this distribution of energy in actual practice, it would likely be necessary to use larger reflectors and to

maintain the 500-watt lamps farther from the area to be illuminated. Because of the differences in transmission and reflection of the various wave lengths, it is doubtful whether the intensity of the visible light would be very different in the use of the two lamps under these conditions. It is apparent that a choice must be made between the use of many lamps of low wattage or fewer lamps of a higher rating. The choice will usually resolve itself into a consideration of the expense of installation and the upkeep of the various types.

In our own work, 200 and 300-watt lamps have been found to be satisfactory as a supplement to sunlight during the winter months. Above each of the center beds are suspended two 1-inch conduits about 1 foot from either side of the bench. Every 4 feet in the conduits there is fitted a receptacle with two outlets for the plugs of lamp cords. The lamps are suspended from the conduits by a light-weight chain which is attached to the shade by means of a yoke, also made of chain. These chain supports are adjustable, so that the position or the height of the lamp can be varied at will. The conical reflecting shades for the 200-watt lamps are 10 inches in diameter at the base and about 3 inches in height; a collar 1.5 inches in height and 2 inches in diameter is fitted to the top of the cone to facilitate attachment to the lamp socket. The shades are of bright tin and are sprayed with a clear lacquer to preserve the reflecting surface. The two middle beds are provided with twenty-eight 200-watt lamps suspended 12 to 18 inches above the plant cultures; each lamp provides illumination for about 4 square feet, and the lights are moved about until the illumination over a bed is as uniform as possible. For the illumination of the side beds, 300-watt lamps 4 feet apart and about 30 inches above the beds are used; these lights are screwed directly into the receptacles, which can be moved through an angle of 45°. In this way a more uniform distribution of the light over the bed is obtained, but the illumination is not nearly so uniform over these side beds as over the center ones. A larger reflector is also necessary; a conical shade 14 inches in diameter at the base and 5.5 inches in height is used.

The advantage of this system of lighting is the flexibility of the set-up. The lamps can be moved whenever necessary, and in this way the intensity of the illumination can be kept fairly constant over the plants. The lights are used between 8 A.M. and 9 P.M. on dull,

cloudy days, but on bright days they are used only after the sun has declined to a point where artificial illumination becomes an effective part of the total radiation. Since the lights are fitted to the receptacles with pull-out plugs, they can easily be removed during the months in which natural illumination is sufficient; that is, from the middle of April to the first of October.

TEMPERATURE.—One of the most difficult factors to control in greenhouse work is temperature. This is especially true during the summer, since most systems of ventilation are useless at this time unless refrigeration of the incoming air is possible. During the winter, steam heat, controlled by a bimetallic regulator, is used to keep the temperature about 25° C. When the lights are in use the temperature rises, but by opening the ventilators in the roof slightly and using large electric fans to keep the air circulated, it is possible to maintain the temperature between 25° and 30° C. without much difficulty.

In early spring, an unusually bright day may send the temperature up 10°–15° C. unless there are means to reduce the intensity of the sunlight coming through the glass. To avoid this overheating it has been found advisable to provide shades for the roof. These are made of "longcloth," 3 feet wide and 12.5 feet in length, and are fastened by means of brass eyelets to heavy copper wire. The shades are easily made and the materials are inexpensive; they are very effective in lowering the intensity of light whenever it becomes so great that the temperature of the greenhouse cannot be kept below 30° C. The shades have an advantage over whitewash in that it is necessary to shade only that part of the greenhouse which is receiving direct illumination, and hence reflected light and skylight can be utilized entirely. As spring advances, however, it is necessary to apply whitewash to the roof of the greenhouse in successive coats; by July, a fairly heavy protection of whitewash has been applied to the upper part of the roof which receives the intense radiation of the midday sun, and a less heavy coat to the remainder. Of course, the application of the whitewash lowers the intensity of illumination; but, because of the long days at this time of the year, small leguminous plants, as clover and alfalfa, grow rapidly and with no evidence of lack of light. For larger plants, as peas and soy beans, it has been found advisable to use the less easily controlled outside cold-frames.

During the spring and summer months the beds and walks are kept wet; 16-inch fans keep the air circulated, which aids in lowering the temperature in the sand beds by rapid evaporation of their moisture. Removal of several panes of glass from the roof at the eaves allows a good circulation of air to be maintained throughout the greenhouse.

ROUTINE CONTROL.—The methods described for the regulation of the various factors require a certain amount of routine checking and analyses for their successful operation. Every two hours during the day an inspection of the house is made, at which time the flow of the gases is checked; the temperature, humidity, nature of illumination, and other observations are recorded in a log-book. This routine check starts at 8 A.M. in the winter and 7 A.M. in the summer; at these hours the gas mixtures are started, the lights turned on if necessary, and the beds and walks watered. The last inspection is made at 9 P.M., at which time the gas mixtures are stopped, the lights are turned off, and the ventilators adjusted. Temperature readings are taken in various parts of the greenhouse on thermometers fitted in the rubber stoppers of the type of bottle shown in figure 2 *b;* the bulb of the thermometer is blackened in order that the indicated temperature will be a maximum. The temperature is maintained between 25° and 30° C. in so far as is possible; if the readings are not within these limits, the ventilators and fans are adjusted to remedy the excess or deficiency.

There is no provision for control of humidity in the greenhouse proper, but readings are taken with a Tycos hygrodeik in order to take corrective means when needed. Usually the humidity is 60 to 70 per cent, and is maintained fairly constant by the watering and the air circulation; it is easier to control during the winter than during the hot, moist summer. The humidity of the gas mixture is regulated to a certain extent by passing the gases over saturated NaCl solution, but this is not too successful, since moisture often collects on the inside of the bottles used to grow the plants. The composition of the gas mixtures is rather easy to regulate, since once adjusted, the rate of flow of the various gases is maintained from 2 to 3 hours without change.

At the time of each inspection, a note is made in the log-book in re-

gard to sunlight and whether or not electric lamps are in use; in this way the total number of hours of artificial illumination can be calculated for a given experiment. Meteorological data from the local Weather Bureau are added to these observations at the end of each month. If possible, routine determinations of the total illumination received at various times of the day might prove of value. The various types of apparatus for measuring light have been discussed by SHIRLEY (**16**), with comment on the accuracy and technical skill required for manipulation. In control of this nature great accuracy is not required, since the intensity of light will vary in different parts of the greenhouse, especially when the illumination from the sun is included. Ease and speed of manipulation are much more important than an error of less than 1 to 2 per cent. A photometer based on the action of a photoelectric cell has recently been put on the market by the Weston Electric Company; it is claimed that it is accurate to 5 per cent when used to measure the illumination from the sun or an electric lamp, and that it is very easy to operate. One has been ordered and will be tested during the next growing season. Measurements made with a photometer of the illumination from the electric lights alone indicated an intensity of 250 to 300 foot-candles at the level of growth of the plants. If no special apparatus for the measurement of light is available, indirect methods such as those described by LIVINGSTON (**11**) might be a desirable substitute.

In addition to these routine observations, certain periodic analyses might be made; for example, CO_2 in the gas mixture and ultraviolet radiation. A simple method for the determination of CO_2 in a mixture has been described elsewhere (**21**). Air plus CO_2 is bubbled through a solution of $NaHCO_3$ of known strength; the pH of the solution is determined by a glass electrode, or colorimetrically; the pCO_2 in the air is determined from a calibration curve by means of this pH.

In addition to the visible light measurements, some idea of the quantity of radiation of lower wave lengths in the illumination is often required. Since the ultraviolet and near ultraviolet light will vary with the period of the year and with the type of artificial light used, a quick convenient method for estimation is desirable for periodic analysis. LUCKIESH (**13**) has reviewed the many methods avail-

able for estimation of ultraviolet radiation; these are based on some specific effect of light of low wave length, such as its photochemical, physical, photogenic, physiological, or photoelectrical action. Accurate measurements require the use of the thermopile, bolometer, or the radiometer with suitable filters, but these methods are too time-consuming and exacting for routine analysis. The most promising methods for general use appear to be those based on the photochemical effects of the ultraviolet and near ultraviolet rays; these are suitable provided it is desired to determine the ultraviolet and violet radiation as a whole rather than any particular narrow range of wave lengths. None of the photochemical methods is specific for a given region (for example, less than 3000 A°), but will overlap, with a changing quantum efficiency, into higher wave lengths. Nevertheless for control work these methods have much to recommend them, provided they are used with full cognizance of the limitations.

A variety of photochemical reactions for the measurements of ultraviolet light have been proposed, including the photochemical decomposition of methylene blue (**9**), of quinone (**5**), triphenyl methane dyes (**20**), and uranyl oxalate (**1**, **10**). The last reaction has been investigated very thoroughly in regard to the effect of different wave lengths, the temperature coefficient and quantum yields, and for this reason can be used with greater confidence than the other reactions. The technique of the estimation has been described in detail by LEIGHTON and FORBES (**10**). These workers showed that wave lengths up to 4900 A° decompose uranyl oxalate, but that the percentage of radiation absorbed decreases rapidly for wave lengths above 4000 A°. They also found that the temperature coefficient of the reaction is only 1.03, and that the quantum yield varies from 0.49 to 0.60 with an average of 0.55. These data indicate that with polychromatic light the method will measure not only the ultraviolet, but to a certain extent the violet and blue; that is, above 4000 A°. This latter is not a serious objection to control analyses, but it should be kept in mind when making measurements; for example, the light from a 200-watt lamp gives some decomposition of the uranyl oxalate, but this is probably due to the presence of wave lengths above 4000 A°. For greenhouse work, special containers for the uranyl oxalate during exposure can be made from small weighing

bottles; these are covered with tinfoil on the outside to reflect the radiation not absorbed and are filled with sealing wax until the surface of 10 cc. of solution is almost level with the top of the bottle. The surface exposed should be about 400 mm.^{2}, so that the depth of liquid is 2.5 cm. This depth is necessary, as ANDERSON and ROBINSON (1) have shown that not all radiation is absorbed unless the thickness of the solution exposed is 1.5–2.0 cm. After 30 to 60 minutes' exposure, the solution in the weighing bottle is washed into a 25×200 mm. pyrex test-tube, heated at 80° C. for 5–10 minutes, then titrated against 0.02N $KMnO_4$. Blanks on the reagents are made by the same procedure, except that the containers are kept in the dark for 30 minutes. In our analyses, the intensity of the radiation was calculated on the assumption that the average wave length measured is 3600 A°; this calculation is arbitrary, and for comparative purposes the results expressed in cc. of $KMnO_4$ decomposed per unit of time are satisfactory.

The method was tested in the greenhouse by measuring the radiation from a General Electric sun-lamp. It was found that very good checks could be obtained on replicate determinations, and that the intensity measured was independent of the time of exposure; for example, five determinations were made on samples exposed from 45 to 240 minutes and the average intensity found was 68 ± 5.9 ergs per sec. per mm.^{2} In another determination in which the solutions were exposed for 30 to 120 minutes to a higher intensity from the sun-lamp, the average value found was 86 ± 3.7. Part of the variation noted is due to inability to place the solutions so that the radiation from the lamp is the same on all surfaces. When the sun-lamp was suspended 84 cm. from the uranyl oxalate solution, an intensity of 39 ergs per sec. per mm.^{2} was observed; while a 300-watt lamp at a distance of 52 cm. had an intensity of 13 ergs per sec. per mm.^{2} Placing the solutions inside the flint glass bottle used for growing the plant cultures lowered the intensity of these low wave lengths to 50 per cent of the values on the outside.

Discussion

The partial control of greenhouse lighting, temperature, gas environment, and humidity here described is necessarily only relative;

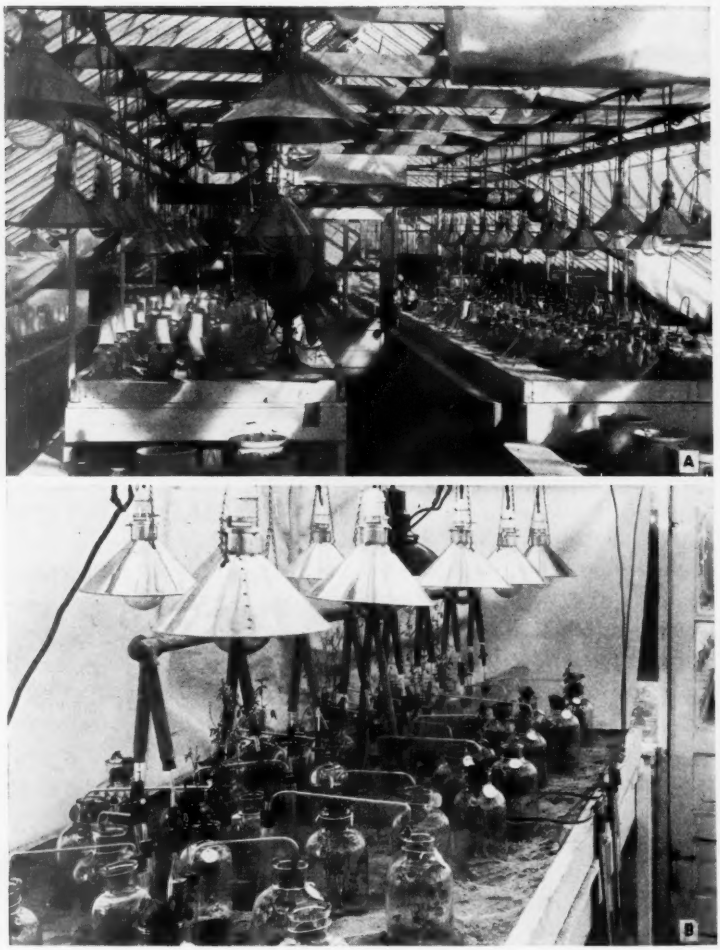


FIG. 3.—*A*, general view of greenhouse; *B*, apparatus for increasing pCO_2 supplied to plants grown in open.

but by a few routine observations and chemical determinations, it is possible to evaluate the environment of a particular experiment to a higher degree of accuracy than is usually obtained in ordinary greenhouse work. In figure 3 *A* is shown a general view of the greenhouse in which these methods of regulation have been applied. Figure 3 *B* is a close view of the system used to grow plants in an open system supplied with additional CO_2. The fine appearance of the plants is obvious in this photograph. The red clover plants, which were about 6 weeks old at the time of the picture, were placed in the greenhouse December 13, and their growth had taken place during a period in which the average daily sunlight was only 2.5 hours. The sweet clover plants which are 3 weeks older are in bloom; about a week after the picture was taken these plants set seed.

Of course the technique touches only a few of the many variable factors which are important in the study of plants, but it is felt that contributions from other workers with like problems will eventually result in a system of greenhouse control that is within the reach of most of the workers in plant physiology or plant chemistry. While the control achieved can never be expected to be more than a first approximation to that developed for small constant-environment chambers, or the larger expensive greenhouse units, it will have the advantage of more universal application.

Summary

1. Methods are described for the partial regulation of the gas, temperature, humidity, and light environment of greenhouse plants. The partial pressure of CO_2 in the gas mixture supplied the plants is regulated by mixing air with CO_2 from a cylinder; the volume of each constituent is measured with a standardized flowmeter. The humidity of the air-CO_2 mixtures is partially controlled by passing these over a saturated solution of sodium chloride.

2. To supplement the natural illumination from the sun with artificial lighting in the winter months, 200 and 300-watt Mazda lamps, equipped with bright tin reflectors, are provided. The lamps are kept 12 to 18 inches above the cultures, and are so distributed that the intensity of the light received by plants is about 250 foot-candles.

3. The temperature of the greenhouse is regulated by means of steam heat, ventilators, electric fans, and frequent sprinkling of the sand beds and walks. In order to reduce the intensity of the sunlight during the spring, the roof of the greenhouse is equipped with shades; during the summer a heavy coat of whitewash is applied to the roof. Commercial copper sulphate is distributed throughout the house to prevent the growth of algae.

4. Routine analyses are made periodically and recorded in a log-book; these include meteorological observations, estimation of CO_2, and determination of ultraviolet light.

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FORMATION OF CALLUS KNOTS ON APPLE GRAFTS AS RELATED TO THE HISTOLOGY OF THE GRAFT UNION[1]

JOHN E. SASS

(WITH PLATES IV–VII)

Introduction

The common occurrence of gall-like overgrowths on apple nursery stock is a source of considerable economic loss to nurserymen. It is now generally known that a certain type of overgrowth is caused by an organism, *Pseudomonas tumefaciens* Sm. and Town. Evidence has been accumulating, however, to show that all overgrowths on grafted apple nursery stock are not necessarily of bacterial origin. As recently summarized and augmented by MUNCIE and SUIT (**9**), it seems that the best isolation technique recovers a pathogen in less than 1 per cent of the overgrowths examined. The great majority of the overgrowths encountered in the nurseries apparently develop in the absence of a pathogen.

Our limited knowledge of the anatomy of true (bacterial) crown-gall and non-bacterial overgrowths has accumulated largely as a secondary issue of bacteriological investigations. It is clear that the tissues of both types of overgrowth consist of proliferated tissues of the plant. In the case of crowngall, the excessive proliferation of the host tissues is brought about by the presence of bacteria in the tissues. In view of the fact that non-pathogenic overgrowths are most common on poorly-healed grafts (RIKER and KEITT **12**, MUNCIE and SUIT **9**), the cause of excessive callusing has been ascribed to some disturbance in the healing process.

HERSE (**5**) made a detailed study of the histology of healing in splice grafts of apple. He showed clearly that the respective calli of

[1] This work has been carried out in connection with the crowngall project in which the Crop Protection Institute of the National Research Council, the University of Wisconsin, the Office of Mycology and Disease Survey of the United States Department of Agriculture, and Iowa State College are cooperating. The manuscript has been critically read by Dr. I. E. MELHUS.

the stock and scion coalesce by the mingling of spongy callus cells, and that in well-matched grafts a new cambium appears, bridging the intervening callus between the members in about ten weeks. In poorly-matched grafts, instead of a straight bridging layer of cambium, a recurved cambial extension is differentiated from the stock and scion respectively. Subsequently there are formed on the margin of each member of the graft irregular and unconnected rolls of vascular tissue, which may constitute more or less permanent barriers to union. An excellent review of the literature on the healing of wounds and graft unions has recently been published by BRADFORD and SITTON (2), making repetition in this paper unnecessary.

Materials and methods

Scion wood for the grafts used in this work was taken from Wealthy trees near Ames, Iowa, and the roots used as stocks were from one-year-old Kansas-grown French crab seedlings. Piece-root tongue grafts were made by Dr. J. H. MUNCIE, in a manner similar to nursery practice except that the cut surfaces were kept reasonably clean, and most of the joints were fitted accurately. Standard waxed twine was used for wrapping. Only the stated combination of stock and scion was used, and one type of graft was made. The data presented in this paper were obtained entirely from this material. The grafts were promptly packed in sterilized peat, in which they were stored at about 18° C. until used. Representative grafts and growing trees were collected at intervals and prepared for histological study.

For the study of the early stages of callusing, thin slices were removed from the callusing surfaces of the separated stock and scion. Just enough wood was included to furnish orientation. The graft unions were removed intact as soon as they had callused enough for the members to stay together during handling. In order to facilitate penetration by reagents, superfluous wood was cut away, leaving only the callusing surfaces involved in the healing process.

Acetic-formalin-alcohol was used as a killing agent. The younger stages were imbedded in paraffin, and the complete grafts were handled in celloidin. Sectioning of the more woody paraffin material was greatly facilitated by treating the blocks, trimmed ready for

cutting, in water at 40° C. for two or three days. After this treatment the paraffin becomes translucent, aiding in orienting in the microtome, and the sapwood and attached bark are sectioned easily. Mayer's haemalum and safranin were found to be the most useful stains.

Early stages of callus formation

The type of graft used in this study is made during the winter, when the tissues are in the dormant condition. At this time there is a clear-cut demarcation between the matured wood of the past season and the tissues of the bark. In this paper the term bark is used to include all tissues outside of a more or less distinct woody cylinder. Dormancy is broken very soon after the grafts are made, for mitotic figures were evident in the tissues of grafts stored at about 18° C. for two days. Activity first becomes evident in tissues of the bark, as shown by mitotic figures (fig. 1), by the general expansion of this region, and by the presence of linear series of cells which have resulted from recent cell divisions (fig. 2). Although the preparation of the tissues was not designed to yield cytological details, the numerous minute chromosomes were readily discerned in metaphase plates. The orientation of the mitotic figures is variable, but the spindle tends to be at right angles to the cut surface, and several successive divisions commonly occur in one plane (figs. 2, 3). If the cut surface is clean, meristematic activity begins in cells on the very surface of the cut (fig. 2). If the surface cells are injured, activity is more likely to begin deeper within the tissues (fig. 3).

Some preparations show early activity in the cambium, and the cambium and the most recently formed secondary phloem may produce most of the callus (figs. 11, 12, 13). However, it has become more and more evident during this study that the cambium may be a minor factor in the formation of callus. The primary cortex and the older secondary phloem, located well outside of the cambium, may in some cases produce most of the callus. In fact, callus may be derived largely from primary cortex of the scion (figs. 5, 12) or from secondary phloem of the stock or scion (figs. 12, 14). The tissues of the bark may contribute variable proportions of the total callus. In this material the periderm does not seem to produce callus. This is particularly evident in figures 4, 6, 12, 31. The extent

of the proliferating area is variable. Only limited areas may produce callus (figs. 10, 11, 12, 13) or nearly the entire cut face of the bark undergoes proliferation (figs. 4, 14, 31).

In the manufacture of grafts by the usual nursery methods, the grafts are frequently subject to conditions which interfere with callusing and healing. If the cut surface of the bark is kept clean and if excessive desiccation does not occur, extensive superficial callusing takes place as just described. But in most of the preparations of early stages examined, there were noted more or less extensive dark areas of dead cells (figs. 4, 11, 12, etc.). These cells were found to be crushed, and grains of sand were abundant on the surface. It is probable that the dead areas were the result of crushing, injury by grit, or drying, somewhere along the process of making and storing the grafts. Regardless of the cause of the formation of these inactive areas, they constitute a barrier to effective contact between the stock and scion. The duration of the barrier action is variable. Active proliferation of the deeper tissues may eventually rupture the dead surface layer (figs. 4, 6, 10, 11). Subsequent coalescence of the stock and scion leaves more or less displaced sheets of dead tissue buried in the callus (figs. 5, 7, 14, 28). In some cases, extensive and more permanent inactive areas seem to retard callusing of the member involved (figs. 11, 12, 13, 15).

The foregoing description of the early stages of callusing takes into account various forms of mechanical damage to the meristematic surface. Since the grafts were not made under aseptic conditions, it is not at all surprising to find that, under conditions of excessive humidity and temperature, fungi gain a foothold on the cut surfaces. The disorganized area in the bark of the scion in figure 9 contains abundant septate mycelium, strands of which extend across the gap to the root. The effect of this invasion and destruction of the tissues obviously prevents proliferation and retards or prevents union in this region between members of the graft.

In clean, well-made grafts, in which the stock and scion are reasonably well matched, unobstructed contact is soon made between the respective calli of the members. The spongy callus cells mingle, and growth pressure compresses the mixed callus until the respective cells of the stock and scion become indistinguishable (figs. 4, 5, 14).

No attempt has been made in the present study to accomplish differential staining of stock and scion cells. After contact is established, the cells on the outer tangential surface of the callus become suberized (figs. 4, 5). Outward radial proliferation is thus effectively stopped, and cell division then becomes confined to the limited exposed surface in the space between members of the graft (figs. 4, 5, 14, etc.). Proliferation continues on this free surface; and in very rare cases, especially near the scion lip and the "shoulder" of the root, the gap between stock and scion may be filled by parenchymatous "filler callus" by the end of the first growing season. In most grafts, however, especially in the median region of the graft union, the space between the members is not filled during the first season.

In the varieties of apple used in this study, no proliferation occurs during the first growing season from the xylem rays, the cut ends of which terminate in the gap between the members (figs. 10, 20, 21, etc.). These wood ray cells, though living, do not undergo division. The other xylem elements and the pith produce no callus whatever. Examination, under higher magnification, of the contact between the filler callus and the contiguous wood ray cells shows clearly the thin-walled spongy callus cells pressed into the cut lumina of the thick-walled xylem ray cells. The filler callus between the woody cylinders, as well as the rest of the callus of the graft, is derived entirely from tissues of the bark.

Cambial bridging between stock and scion

If the stock and scion are radially matched along a particular edge of the cut the respective cambia are practically on the same arc, and the tangential gap may be filled by callus in less than two weeks. With a larger gap a longer period may be required (fig. 12); but in any reasonably well-matched and clean gap, unobstructed mingling occurs between the spongy, undifferentiated callus cells derived from the stock and scion respectively (figs. 4, 5, 14). Although the cells derived from the stock and scion soon become indistinguishable, it is evident from the early stages that the stock and scion do not necessarily contribute equal quantities of callus. In fact, most of the callus in the gap may have been produced by one or the other member.

After the gap between the cambia becomes filled with callus, the formation of a "bridging cambium" begins. Along the cut edges of the old cambium, the adjacent callus cells undergo several divisions radially, producing several layers of narrow, tangentially elongated cambium-like cells. This activity extends tangentially to callus cells farther away from the respective cambia of the stock and scion. The callus between the members is finally "bridged" by a distinct, cambium-like layer of deeply staining cells with large nuclei (figs. 7, 8). In view of the structure and subsequent activity of these cells, they constitute a true cambial region. The important features of the development of this new cambium are that the cells differentiate from spongy callus, that re-activation begins adjacent to the old cambium, and that differentiation progresses tangentially until a cambial arc connects the respective cambia of the stock and scion. The period required to achieve this bridging is very variable, but many good cases of successful cambial union were noted in grafts three weeks old.

Effect of accurate fitting of the graft on the healing process

Perhaps the most important factor in producing successful grafts is the fitting of the joint between the stock and scion. Although it is generally known that a properly made graft is more likely to make a good tree than is a poor union, the exact histological details of the failure of healing have not been clearly understood. In order to emphasize the desirability of making good grafts, the comparative histology of good and poor unions has received particular attention in this study.

The most common type of misfit in piece-root tongue grafts is the result of combining a small scion and a large root. The root is often 50 per cent larger than the scion. Occasionally the scion, regardless of its relative size, is so placed that its edge projects over the edge of the root. If the cambium of one member of the graft falls outside of the cambium but within the periderm of the other member, the respective calli can make contact, the extent and effectiveness of the contact depending on the degree of overlapping (figs. 10, 14, 16). The mixed callus, derived from both members, as shown in figures 10 and 14, proliferates inward as well as outward. On the outside

margin of the cleft the exposed callus proliferates rapidly, at first by the division of surface cells, and later by the division of cells below the surface, building up ridges and irregular masses of callus. In figure 16 the entire cut surface of the root seems to have been inactive, and the entire callus was produced by the scion. In this case the new cambium differentiated from the callus, instead of continuing the cambial arc, tends to be curved inward (fig. 16 *b*) or outward, and obviously cambial bridging between the stock and scion is delayed or even prevented.

If the cambium of one member falls radially outside of the periderm of the other member, an exposed callus proliferates from the active tissues of the protruding member (figs. 11, 13, 15, etc.). The free callus presently comes into contact mainly with the cork of the recessed member. Such contact is highly ineffective from the standpoint of establishing a successful graft union. In the graft shown in figure 12, the periderm and cortex of the recessed scion had been cut away. The exposed tissues of the scion had proliferated outward, while the cambium and secondary phloem of the root proliferated inward. Contact between the calli may often take place in this way.

The foregoing description of cambial bridging has taken no account of secondary activity in the old cambium of the graft. Since callusing follows the breaking of dormancy, a corresponding reactivation of the dormant old cambium is to be expected during the period of callusing. The cells of the old cambium begin to divide several days after the grafts are made, and in ten days an appreciable increment of xylem is laid down (fig. 6). This cambial activity seems to aid in rupturing any barrier layer of dead tissue (fig. 14 *a*) in the vicinity of the cambium, and probably facilitates early cambial bridging.

Progress of healing after cambial bridging

During the three weeks' interval just described, the behavior of the callus along the margin of the cleft and in the gap between the members is variable, depending on the matching of the edges. The inward proliferation of filler callus progresses by the division of the spongy cells on the inner surface of the callus (figs. 4, 14). The cells of the callus between the xylem cylinders of the members do not differentiate into vascular elements during the first season. Inward growth of the callus is soon terminated by the formation of a suber-

ized layer on the inner surface. Figure 18 shows this limiting layer to be well developed in twelve weeks. The remaining gap probably does not become subsequently filled with callus.

The development of the marginal callus along the outer edges of the union is much more influenced by the matching of the members than is the history of the filler callus. On a well-matched edge very little callus is formed beyond the edge of the cleft. The surface cells become suberized and further outward proliferation is effectively stopped (figs. 5, 7, 27 *a*). In a poorly-matched graft there is known to be a tendency to produce excess callus (figs. 10, 11, 13, 15, 27 *b*, etc.). This condition is associated with delayed cambial bridging. At first the free marginal callus consists of undifferentiated parenchyma. Presently there appears a concentric zonation of the cells, until a well-defined cambium-like zone develops (fig. 13). Centrifugal expansion of the excess marginal callus progresses by tangential cell divisions in this meristematic zone. In the older central regions of the callus, some of the cells differentiate into contorted xylem elements. Further work is now under way on the histology of the continued growth, internal differentiation, and ultimate fate of excess callus.

In a well-matched graft, the establishing of transverse and vertical cambial continuity produces a complete layer of cambium over the union. As previously described, typical cambial activity begins in the newly differentiated cambium as well as in the old cambium. It has long been known that after this point, continued normal activity of the cambial sheath adds successive increments to the vascular cylinder during the life of the tree. Transverse sections through representative "good" trees (over 3 feet high) during the first season show good vascular continuity on all or most of the six planes of contact (figs. 19, 27). This condition is described in more detail in the legends of the illustrations and hence requires no extended description here.

The histological details of the course of development have been less clearly understood in the case of poor unions than in good unions. As shown in figure 16, a recurved cambial extension is differentiated from the callus in a poorly-matched graft. This may occur even in a fairly good match (fig. 17). Typical cambial activity takes place in

this new cambium as well as in the original cambium of the member. The result of this activity is the laying down by the old cambium of a normal xylem increment to the original xylem cylinder, while the new, incurved or outcurved cambium lays down a partial cylinder or even an irregular bar of xylem on the margin of the member (figs. 20, 21, 22). Thus there is no continuity between the respective xylem increments of the stock and scion, the only contact being between the proliferated calli of the adjacent members. In some grafts, the parenchymatous area between the members is eventually bridged by cambium differentiated from the callus, and a continuous vascular cylinder is subsequently laid down.

Summing up briefly the comparative histology of successful and unsuccessful unions, it seems that the relative size and vigor of the grafted tree by the end of its first growing season are largely conditioned by the continuity of the second annual ring of the tree, laid down during the current growing season. Changes occurring in the first annual ring (the original stem and root used in making the grafts) probably have no influence on the development of a successful union.

Formation of callus knots on the scion lip

One of the principal objectives of this study has been to trace the origin and development of the gall-like knots which occur on the scion lip. The orientation of the scion lip with respect to the adjacent root is highly variable in the general run of grafts. At one extreme the oblique face of the short scion lip does not cover the cut on the root. Consequently the cut tissues of the bark of the scion are in contact with the wood of the root, while the cut bark tissues of the root are exposed. At the other extreme the long scion lip extends beyond the corresponding face of the root; hence the cut bark of the root may be in contact with scion wood, and there is an exposed area on the under side of the scion lip. Between these extremes all intermediate conditions are found.

Since it is impossible to study the anatomy of a given underground knot through the season, collections for this study were made at intervals during the season, selecting representative "good" trees

over 3 feet high, and "poor" trees less than 1 foot high. Obviously the presence or absence of overgrowths was not known before digging. There was found to be much variation in size and degree of differentiation in overgrowths of a given collection. In the present paper, emphasis is placed on the comparative histological relationships in the union in thrifty and in poor trees, and in knot-free and knotty trees.

A radial section through the scion lip ten days after grafting shows clearly that any or all living tissues of the bark, excepting the periderm, may produce callus (fig. 31). The progress of the wound reaction and the tissues involved are the same in the case of the scion lip as in other parts of the union. In view of these similarities, the matching of the scion lip may be expected to bear some relation to excessive callusing on the scion lip.

Figure 32 shows a representative case in which a knot had developed on a "short" scion lip, in which the tip of the scion lip was in contact with the wood of a relatively large root. Obviously the callus of the scion lip was not in contact with root callus, and as in the cases of lateral misfits, excessive callusing occurred on the scion lip. The knot illustrated had developed in three weeks in storage. The large knot shown in figure 35 developed on a long, overlapping scion lip. Since the active tissues of the scion lip were not in contact with callusing tissues of the stock, coalescence of respective calli did not occur, and excessive callusing of the free scion lip formed a knot. Some vascularization of the knot tissues had taken place, as shown by the irregular zone of xylem (*d*).

It is generally believed that the lip knot consists entirely of scion tissue, and this is probably true in many cases. The present work, however, has shown that as in the formation of excess marginal callus, both stock and scion may contribute to the callus of the lip knot. The bark on the cut faces of the root produces some callus, and if this callus comes into unobstructed contact with the callus of the scion lip, some degree of coalescence occurs. Since the rate of callusing is greater on the free member, the scion, the bulk of the knot is scion callus. But as shown in figure 34, more or less stock (root) callus may become incorporated into the knot. From the

standpoint of the possible origin of bud primordia in the callus, it should be borne in mind that the callus may consist of both stock and scion tissue.

Knots are known to occur on the scion lip in large, well developed apple trees, although this is contrary to the usual rule. In the absence of isolation data it is not improbable that in these field-grown trees some of the older knots may be crowngall, but histological study shows that the knot on the scion lip of a vigorous tree is usually associated with poor healing of the lip joint, whereas the rest of the union is properly healed. In this case the tree as a whole may become vigorous, but a large knot may nevertheless develop on the scion lip.

This work has shown that local regions of the graft union may react more or less independently in the development of excess callus on various parts of the union. The vigor of the grafted tree and the formation of excess callus are both determined, though independently, by the vascular effectiveness of the tissues laid down incident to healing. Non-pathogenic overgrowths are masses of excess callus, and their development is determined by the conditions which influence the phenomena of callusing.

Discussion

Of the various possible stimuli which may initiate the processes of repair, the most obvious are external mechanical forces. SMITH (**13**), however, has shown that proliferation can be induced in the pith of *Ricinus* by the use of chemicals, entirely without mechanical means. It seems that mechanical forces are not necessarily the exclusive stimulatory agencies, and we may look for some factor within the tissues.

A possible internal agency may be sought in the food relationships in the organs involved. KÜSTER (**6**) and others have stated that organs containing abundant food, callus more extensively than organs having little food. In stored and comparatively inactive apple grafts, however, callus knots are generally more prominent on the scion, in which there is relatively less food, than on the root, which is literally full of starch. This is not in harmony with KÜSTER'S observations. Perhaps callusing is influenced by the alteration of

stored food following dormancy rather than by the quantity of food present.

Photosynthesis can certainly be eliminated as a factor during the early stages of callus-knot formation prior to planting in the field. However, RIKER and KEITT (**12**) and MUNCIE (**8**) have shown that callus knots are associated with poor graft unions on growing trees; and it seems probable from the present histological study that, during the growing season, lack of vascular continuity brings about the accumulation of the products of photosynthesis and favors the continued enlargement of callus knots.

The rôle of the cambium seems to be somewhat over-emphasized in the literature, particularly in textbooks.[2] BRADFORD and SITTON (**2**) state that pith cells and xylem ray cells (of apple?) produce callus. These writers did not state the source of this information and it is not clear whether they refer to matured ray cells or to recent cambial derivatives. Figures by BAILEY (**1**) and COE (**3**) show that in bud grafts, which are made at a time of rapid cambial activity, the partly differentiated cells of the new xylem proliferate to contribute some of the wound callus. The present work does not support FISK'S (**4**) statement that wood rays and wood parenchyma produce wound callus.[3] At least in the varieties of apple studied, wound callus is produced only by tissues of the bark, that is, the region outside of the xylem cylinder.

Although the necessity of vascular continuity between the members of a graft is clearly understood, there seems to be an impression among nurserymen and others interested in grafting that the cavity (fig. 24, etc.) between the members is responsible for poor conduction, and that the filling in and vascularization of this cavity would improve conduction. MUNCIE and BERKHOUT (**7**) have found a significant reduction in the rate of flow of water through defective

[2] HOLMAN and ROBBINS, Textbook of general botany. 1924: ". . . . the cambium is stimulated to active growth and forms a mass of large, thin-walled cells known as callus." EAMES and MCDANIELS, An introduction to plant anatomy. 1925: "Callus may be formed by the division of parenchyma cells in the phloem and cortex, but its most frequent source is the cambium."

[3] In view of the fact that several investigations dealing with the histology of the graft union have been made in the Botany Department of Iowa State College, the unpublished manuscripts, available at the library of this college, are cited in this paper.

unions. The cause of this poor flow is not the failure of vascularization between the original xylem cylinders of the members, but the failure to lay down an effective vascular ring during the first growing season of the union. In connection with studies on compatibility, PROEBSTING (**10, 11**) has shown that in numerous incompatible interspecific grafts in *Prunus* and *Pyrus* the new tissues between the members are parenchymatous. PROEBSTING attributes unsuccessful grafts to failure to form vascular tissues connecting the members, but he does not state clearly what he means by "vascularization." The present data concerning the process of "cambial bridging" and the subsequent laying down of a vascular sheath are applicable to the morphological aspects of compatibility.

Associated with the general problem of continuity between the members are such problems as protoplasmic continuity, and the matching of pits between adjacent cells of stock and scion respectively. It seems that some workers have not distinguished clearly between plasmodesma and pits. Illustrations by COE (**3**) and BAILEY (**1**), represented as being plasmodesma, undoubtedly represent simple pits and highly reduced full-bordered pits. An adequate technique for approaching these problems has not yet been developed.

The present study has a direct bearing on the crowngall problem. Since the grafts used were not handled under aseptic conditions, the presence of various organisms must be accepted. Furthermore, after grafts are planted in the field they are subject to contact with the soil flora, and the subsequent entry of organisms into the tissues is not precluded. But from this study of the progress of callusing taking place in sterilized peat, from the earliest mitoses in the graft to the formation of large knots, it is probable that at least the early stages of callusing, as here described, represent the condition in non-bacterial overgrowths. These non-pathogenic overgrowths are over-developments of the callusing incident to healing, brought about by some disturbance in the healing process.

Comparing the histological development of these non-pathogenic overgrowths with the histology of crowngall, as described in the literature, largely on plants other than apple, shows several basic similarities in the development of the two types of overgrowth.

Further anatomical comparison of overgrowths, shown by isolation studies to be either bacterial galls or non-pathogenic callus knots, may reveal reliable contrasting criteria for distinguishing between the two types of overgrowth.

Summary

1. The histology of the healing of apple grafts during the first growing season is described, with special emphasis on the formation of overgrowths known as callus knots. Piece-root tongue grafts of Wealthy scion on French crab root were used.

2. Callus is produced exclusively by tissues located outside of the xylem cylinder. Any living tissue of the bark, excluding the periderm, may proliferate. The cambium may contribute very little of the callus.

3. Proliferation is inhibited locally by crushing of the surface cells, by desiccation, or by destruction of the tissues by fungi.

4. The gap between the respective xylem cylinders of the stock and scion does not become filled with callus during the first season. Indications are that the gap does not become filled during subsequent years. The limited amount of parenchymatous filler callus formed does not become differentiated into vascular tissues during the first year.

5. Unobstructed contact between the respective calli of the stock and scion favors a mingling of the parenchymatous cells, and the continued proliferation of a mixed callus. No method has been found for distinguishing microscopically between stock and scion cells in the mixed callus.

6. In well-matched grafts, an arc of cambium, continuous with the respective cambia of the stock and scion, is differentiated from cells of the intervening callus. The complete cambial layer sheathing the union subsequently lays down a vascular cylinder, which comprises the second annual ring of the tree.

7. The size and vigor of the tree at the end of the first growing season are conditioned by the degree of effective continuity in the new vascular sheath around the union.

8. Excess callus is proliferated wherever there is a local obstruction to effective vascular union between the stock and scion. Large

masses of callus, known as callus knots, develop on the scion lip as the result of obstructed union between the scion lip and the stock.

9. Localized callusing may be independent of the general vigor of the tree. Histological interpretations are suggested for the presence of large lip knots on good trees, and the absence of pronounced knots on poor trees.

10. The size and permanence of lip knots or other masses of excess callus are conditioned by the extent and permanence of obstructions to vascular union.

11. Certain phenomena that have been attributed to incompatibility may be interpreted as the result of poor grafting.

12. The internal differentiation of masses of non-pathogenic excess callus resembles somewhat the previously described histology of crowngall.

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EXPLANATION OF PLATES IV–VII

PLATE IV

Figs. 1–8.—Fig. 1, mitotic telophase in primary cortex of scion lip. Regions of graft from which sections were taken are indicated in the sketches. ×500. Fig. 2, stock (root) of graft 2 days after grafts were put into warm storage. Indications of recent cell divisions at *a;* expansion of cells in area *b* prior to division. ×45. Fig. 3, pericycle and primary cortex of scion; surface layer of dead cells (*a*) through which the spongy callus cells (*b*) have proliferated. Meristematic, cambium-like layer has differentiated parallel to surface. Graft 10 days old. ×100. Fig. 4, well-matched edge of a graft 3 weeks old. Scion callus derived from entire cut face of bark, excepting periderm. Dead surface layer (*a*) which had covered original cut face of the member pushed out by callus. Root callus produced by secondary phloem and cambium. Small mass of callus (*b*) has pushed through the obstructing layer (*c*). ×22. Fig. 5, matched graft 3 weeks old. Inactive surface layer (*a*) limits proliferation. Mixed callus (*b*) is the beginning of "filler callus" between the xylem cylinders of the members. ×28. Fig. 6, edge of graft 10 days old (note almost complete absence of callus along plane of contact). Appreciable increment of xylem and phloem had already been laid down in 10 days. Cambia of members not yet connected. ×28. Fig. 7, cambial bridging in well-matched graft 3 weeks old: *a*, inactive surface layer on original cut face; *b*, filler callus derived largely from scion; *c*, dead tissue limiting outward callusing. Note deeply stained layer of bridging cambium. ×18. Fig. 8, enlarged section of cambial bridge. ×100.

PLATE V

Figs. 9–18.—Fig. 9, moldy graft 3 weeks old: *a*, disorganized area producing no callus; *b*, strands of septate mycelium. ×28. Fig. 10, graft 10 days old: *a*, dead layer ruptured by scion callus (*b*); *c*, filler callus produced entirely by cambium and secondary phloem of stock. ×22. Fig. 11, poorly-matched graft 3 weeks old. Extensive dead area (*a*) on stock. Excess marginal callus (*b*) produced where root callus did not coalesce with scion callus. ×22. Fig. 12, scion callus derived from primary cortex which may eventually coalesce with root callus. Graft 3 weeks old. ×18. Fig. 13, some coalescence of stock and scion calli occurring in graft 3 weeks old. Internal differentiation of excess callus begun. ×12. Fig. 14, graft 3 weeks old, showing displacement of strip of dead barrier tissue (*a*) which had been continuous with (*b*). Filler callus (*c*) is a mixed callus derived from both the stock and scion. ×22. Fig. 15, excess callus produced on margin of stock in 3 weeks. ×20. Fig. 16, a poor graft 3 weeks old. Callus produced by scion only. Excess callus (*a*) derived from primary cortex; filler callus (*c*) produced by cambium. Recurved cambial layer (*b*) has differentiated in the callus. ×18. Fig. 17, recurved, new cambium in stock callus and in mixed callus (note absence of proliferation from pith of scion, *a*). Graft 3 weeks old. ×18. Fig. 18, graft dug after

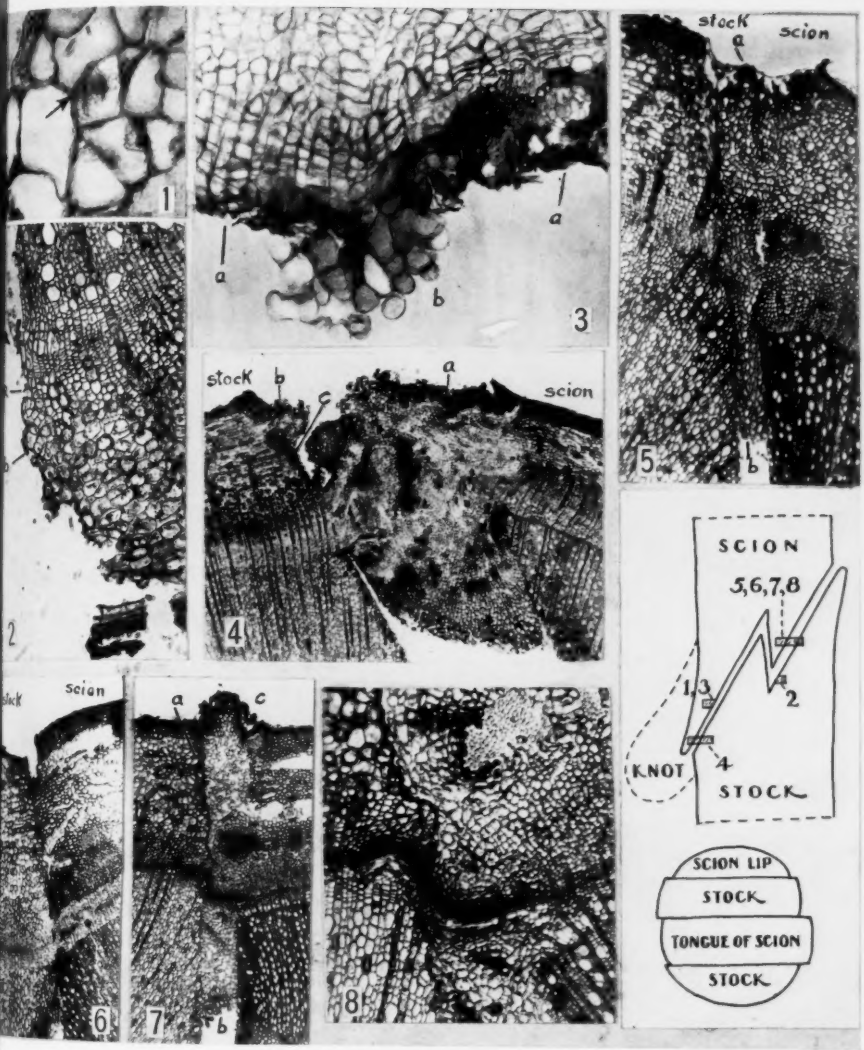
12 weeks in the field (note rolled-in ridge of xylem on edge of each member, produced by activity of recurved cambium shown in fig. 17). Present cambium is nearly straight. Suberized layer (*a*) limits inward proliferation of filler callus. ×12.

PLATE VI

FIGS. 19–27.—Fig. 19, tree 3 ft. high, having a small lip knot, dug after 12 weeks in field. Xylem cylinder of the current season is practically continuous. Callus does not completely fill cavity between original members of graft. ×5. Fig. 20, very poor tree, dormant. Scion cleft has healed on sides *a* and *b*, and partly at *c*. On side *d* a bar of vascular tissue has been laid down by the recurved cambium. Cf. narrow annual ring with fig. 19. ×5. Fig. 21, small knotty tree, showing almost completely separated scion lip. On side *a*, vascular continuity is much better than on side *b*. ×5. Fig. 22, small dormant tree. Marginal layers of vascular tissue *a* and *b* are not in continuity with adjacent member. Root cleft has healed over at *c*. ×5. Fig. 23, very small tree (less than 1 ft. high) having a large lip knot. Side *a* is fairly well healed; on side *b* there is no vascular continuity; on side *c*, continuity is afforded only through parenchymatous tissues. ×5. Fig. 24, small dormant tree. Owing to defective matching, cambial continuity was established late in season. ×5. Fig. 25, very poor, knotty tree at end of first season. Note almost complete lack of vascular continuity throughout the union. ×4. Fig. 26, small knotty tree, having a lip knot on side *a*. Other planes of contact also poorly healed. Negligible amount of callus (*b*) proliferated from cut end of a broad parenchymatous ray of root (this condition is unusual). ×6. Fig. 27, good tree in which the new annual ring is fairly continuous on side *a* and on part of side *b*. Owing to a poor fit on one edge of side *b*, cambial bridging did not occur and a large mass of excess callus had developed. ×5.

PLATE VII

FIGS. 28–36.—Fig. 28, poor tree in which the scion lip is partly separated from the root by layer of dead tissue and a gap at *a*. Members are connected only by parenchyma (*b*). ×6. Fig. 29, mass of callus at *a* was produced mainly by secondary phloem of stock; callus *b* was produced by cambium of the stock. Pressure on the uncut bark of stock has induced proliferation in primary cortex (*c*). ×20. Fig. 30, edge of scion lip on graft 5 weeks old. Scion has produced a mass of callus (*a*), which had proliferated inward at *b*. Effective contact prevented by the area of dead tissue. Small knot present below this level. ×12. Fig. 31, callus knot developed on scion lip in 3 weeks. No proliferation had occurred in the periderm *a* and the wood *b*. ×18. Fig. 32, lip knot 3 weeks old. Callus (*a*) was formed by the proliferation inward of bark callus. In area *b*, some differentiation of callus into contorted xylem elements had taken place. ×12. Fig. 33, scion lip knot from tree 3 ft. high, dug in August. Region of callus (*a*) derived from the stock has coalesced with scion tissue (*b*) of knot. ×4. Fig. 34, lip knot 12 weeks old. Layer of xylem (*a*) is stock tissue; area (*b*) of the knot is stock (root) tissue. ×4. Fig. 35, lip knot 3 weeks old. Tissue *a* was derived from secondary phloem (*b*) of root. Coalescence with the scion knot may readily occur. Note absence of proliferation on the wood (*c*). At *d*, the knot tissues have differentiated into xylem. ×3. Fig. 36, detail of tangential section at margin of lip knot. Enlargement is brought about by activity of layers and whorls of meristematic tissue. Centers of the older whorls differentiate into contorted xylem elements. ×45.



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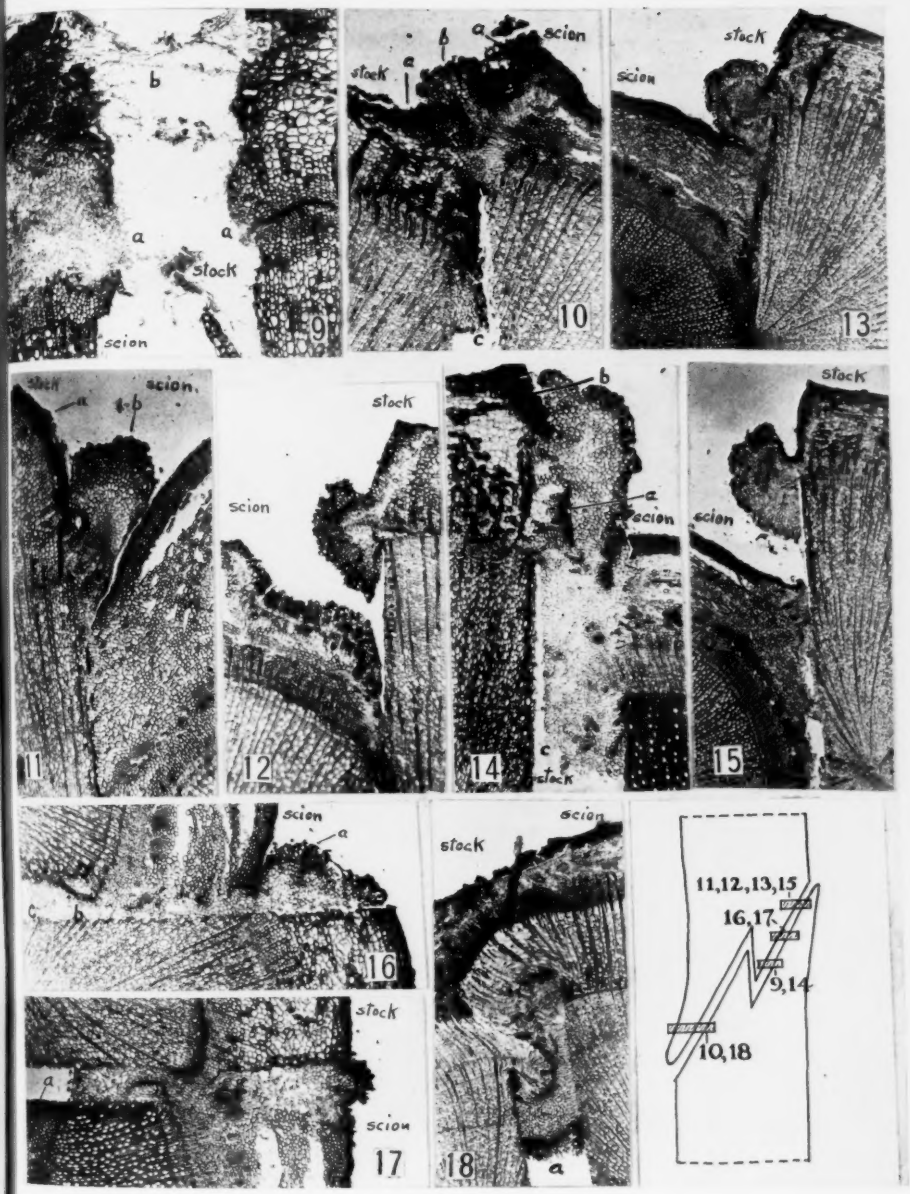

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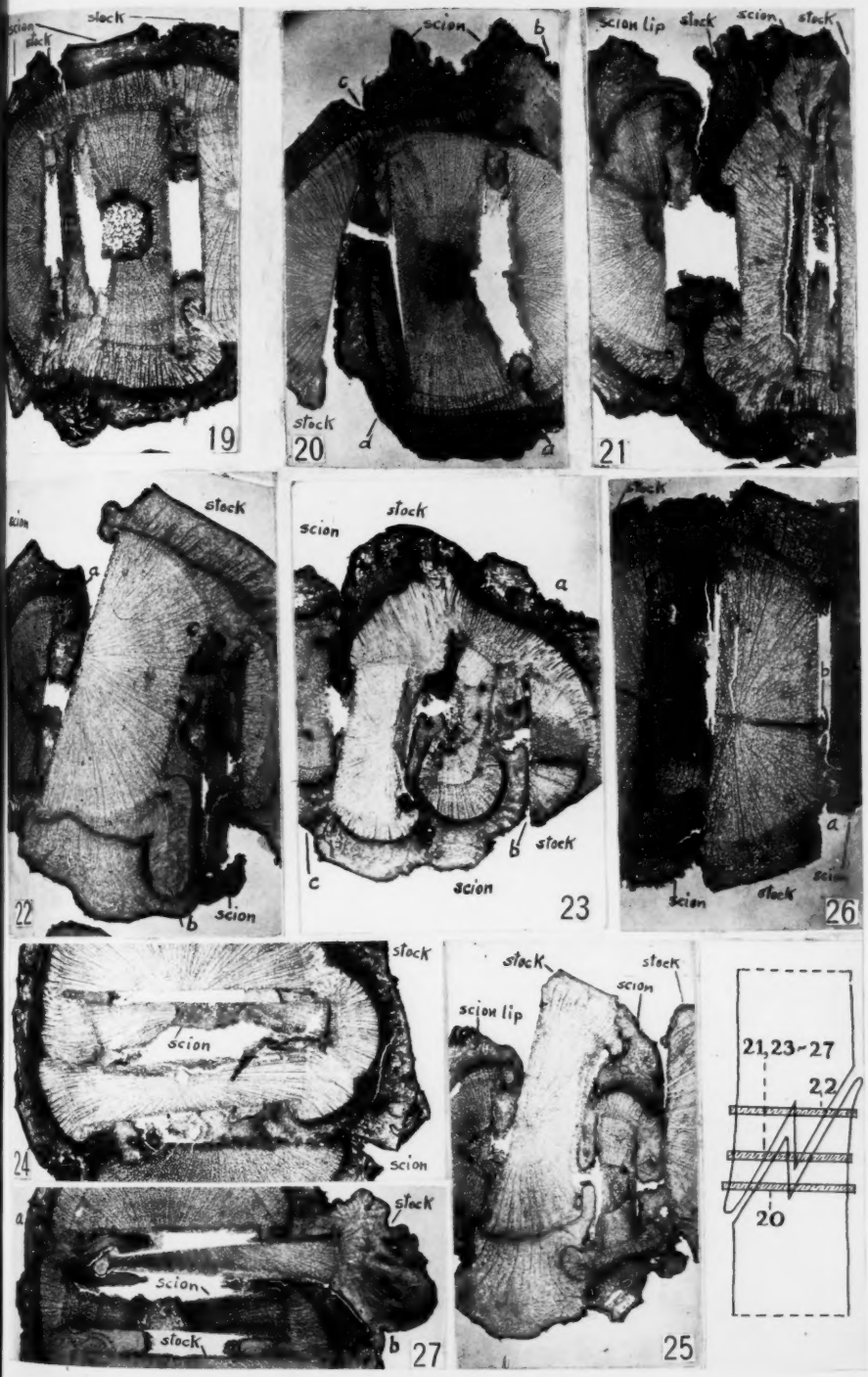

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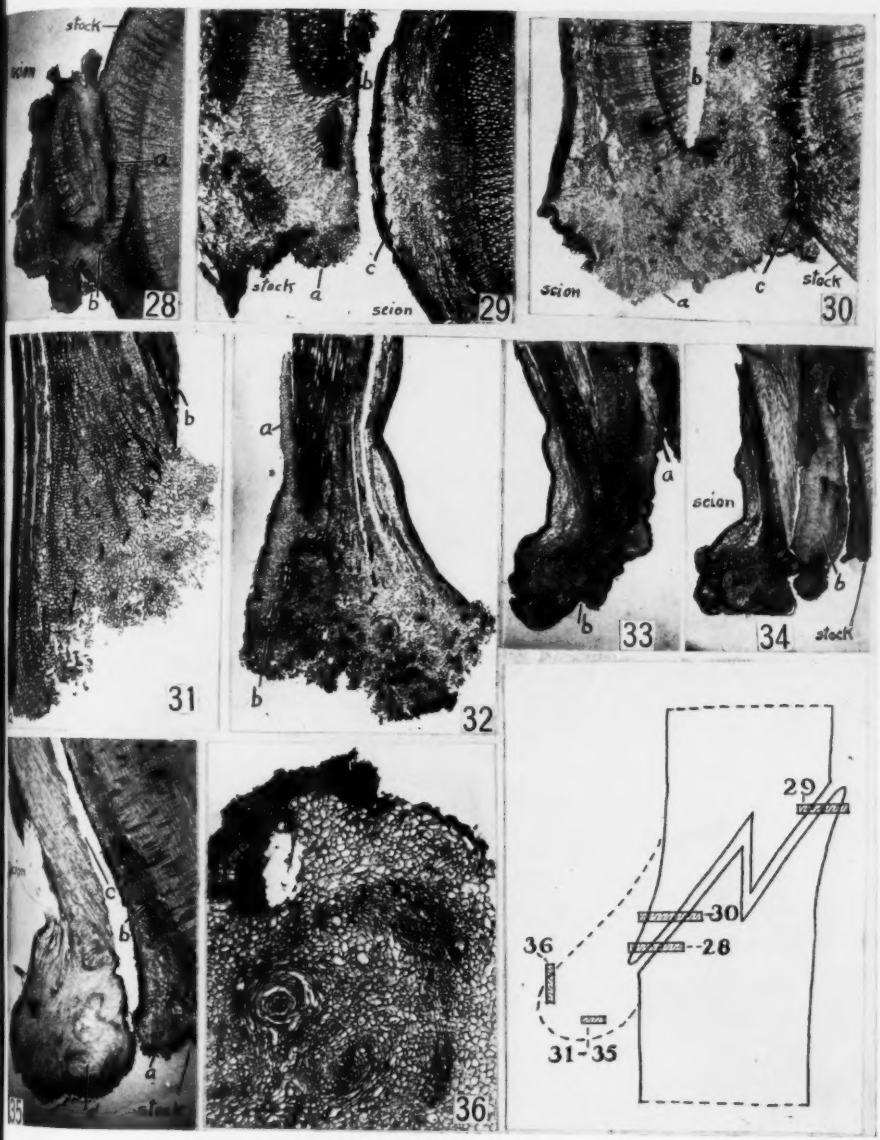

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MINERAL AND NITROGEN CONTENT OF THE LEAVES OF SOME FOREST TREES AT DIFFERENT TIMES IN THE GROWING SEASON[1]

J. S. McHargue and W. R. Roy

Introduction

The mineral and nitrogenous residue which resulted from the annual deposition and decay of forest leaves during prehistoric times was the principal source of the fertility of virgin soils. After the arrival of European settlers in this country magnificent forests began to be cleared away, and the reserve fertility which had been accumulating from decaying leaves and trees through the past ages began to be drawn upon in the processes of leaching, erosion, and the growing of farm crops. During the 300 years in which the art of agriculture has been practiced in this country, considerable areas of land, within the humid regions, have been abandoned for purposes of cultivation because the soils have been so depleted of their virgin fertility that it is no longer possible to produce farm crops upon them. In recent years there has been considerable discussion concerning the reclamation of abandoned farm lands through the process of reforestation.

The growth of important forest trees to a productive state is a slow process under the most favorable conditions. Accordingly, any information that contributes to a more adequate understanding of the kind and amount of the mineral nutrients necessary for the most rapid growth of forest trees may be of some practical and economic importance in the process of reforestation and the conservation of abandoned farm lands.

Serex (5) analyzed leaves collected in the spring and autumn from certain important species of forest trees, and from the results

[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

of his analyses arrived at the conclusion that the average fertilizer value of a ton of forest leaves was approximately $5. TSCHERMAK (6) studied the agricultural value of forest litter and concluded that the absorptive power of the litter increases rapidly with the degree of decomposition, and that its fertilizing value is due to the nitrogen and mineral matter contained in the annual crop of forest leaves.

SEIDEN (4), in a study of the external factors that affect the ash content of plants, found that the ash content of the leaves of trees decreases from the bottom toward the top. He emphasizes the importance of taking leaves from the same part of the plant for comparative analyses. He concludes that the ash content of leaves tends to vary with the amount of water transpired, and that, in the fall, plant nutrients tend to migrate back into the branches. QUARTAROLI (3) reports that the manganese content of leaves increases with the age of the leaf, whereas the copper content decreases. Furthermore, he suggests that copper is concerned in plant metabolism, possibly in a way similar to the vitamins in animal metabolism.

For some time the senior writer and co-workers have been interested in the occurrence, distribution, and probable function of some of the less common elements in plants and animals. In previous reports (2) it has been shown that manganese, copper, zinc, and boron have important functions in the metabolism of plants and animals. This paper reports further data on the occurrence of manganese, copper, and zinc in the leaves of 23 species of trees, and also the percentages of ash, silica, iron, calcium, magnesium, phosphorus, potassium, sodium, sulphur, nitrogen, fat, and crude fiber. The leaves were collected at three different times during the growing season of 1931, from trees growing near the Kentucky Agricultural Experiment Station, in soil derived from a limestone formation. The first set of samples was collected May 4th to 8th, when the leaves were about half grown; the second, August 3rd to 6th, about midseason; and the third, September 4th to 6th, a short time before frost. About three pounds of normal green leaves were gathered from different places and heights on the same trees each time. The leaves of the elm, black oak, and black walnut were somewhat smaller than half-grown when the first samples were taken. The mulberry tree was sampled only in September.

Experimental work

After weighing, the fresh leaves were thoroughly air-dried at room temperature, re-weighed, ground, and put into air-tight containers for analysis. One hundred grams were weighed into silica dishes and heated over the low flame of a Bunsen burner until the easily volatile matter was expelled, after which the dishes containing the partly ashed residues were transferred to an electric muffle furnace and the ashing was finished at about 600° C. The dishes containing the ashes were allowed to cool in desiccators and weighed. The ash was moistened with distilled water, the dish covered with a watch glass, and 1–1 hydrochloric acid run under the watch glass through the lip of the dish until the carbonates were decomposed and 5 ml. excess of the acid had been added. The watch glass was rinsed into the dish, the latter transferred to a hot-water bath, and the contents brought to dryness, after which the residue was baked on a sand bath until all the odor of hydrochloric acid was expelled. The dish was allowed to cool, the residue moistened with 5 ml. of 1–1 hydrochloric acid and about 25 ml. of hot water, and digested on the hot-water bath. An excess of hydrochloric acid at this point is to be avoided because too much acid will interfere with the precipitation of the small amount of copper present; therefore just enough dilute 1–1 hydrochloric acid to bring the acid-soluble salts into solution is all that is necessary. The silica was filtered on an ashless filter paper, thoroughly washed with hot distilled water, dried, ignited, and weighed. The filtrate was transferred to a 150 ml. Erlenmeyer flask and made to a volume of about 100 ml., heated to near the boiling point, and a slow stream of hydrogen sulphide gas bubbled through the solution for about 15 minutes while the flask was rotated, after which the flask was stoppered tightly and set aside for several hours, preferably over night or until the dark-colored precipitate had settled completely and the supernatant solution was clear. The precipitate of copper sulphide was filtered on a pad of paper pulp held in a Caldwell crucible, and washed with a dilute solution of hydrochloric acid which was saturated with hydrogen sulphide, transferred to a porcelain crucible and ignited to the oxide, cooled, dissolved in a few drops of nitric acid, and copper determined colorimetrically by the Xanthate method. The filtrate

TABLE I

ANALYSES OF LEAVES OF 23 SPECIES OF TREES COLLECTED AT THREE INTERVALS DURING GROWING SEASON; RESULTS CALCULATED AS PERCENTAGES OF MOISTURE-FREE MATERIAL

SPECIES	SAMPLE NO.	ASH (CRUDE)	INSOLUBLE RESIDUE (SiO_2)	COPPER (Cu)	IRON (Fe)	MANGANESE (Mn)	ZINC (Zn)	CALCIUM (Ca)	MAGNESIUM (Mg)	PHOSPHORUS (P)	POTASSIUM (K)	SODIUM (Na)	SULPHUR (S)	NITROGEN (N)	PROTEIN (N × 6.25)	FAT (ETHER EXTRACT)	CRUDE FIBER
Pyrus coronaria (crabapple)	1	5.87	0.200	.00080	.0180	.0020	.0007	1.27	0.23	0.34	1.79	0.10	0.19	2.16	13.5	14.9	10.6
	2	6.54	0.340	.00080	.0310	.0020	.0052	1.92	0.32	0.39	1.42	0.04	0.21	1.50	9.4	10.3	11.9
	3	7.92	0.370	.00090	.0300	.0030	.0028	1.84	0.25	0.56	1.54	0.10	0.16	1.21	7.6	12.6	9.9
Fraxinus quadrangulata (ash)	1	6.51	0.340	.00160	.0280	.0060	.0012	0.63	0.43	0.61	2.04	0.40	0.38	5.45	34.1	6.6	9.0
	2	7.12	0.880	.00110	.0330	.0070	.0032	1.91	0.34	0.35	1.32	0.05	0.39	2.46	15.4	3.6	22.4
	3	8.43	1.090	.00130	.0250	.0080	.0034	1.98	0.30	0.42	1.26	0.29	0.11	2.10	13.1	4.1	18.7
Aesculus glabra (buckeye)	1	8.74	0.340	.00050	.0430	.0070	.0035	1.57	0.46	0.61	2.55	0.32	0.31	4.11	25.7	2.9	12.5
	2	10.27	1.410	.00070	.0500	.0100	.0034	3.66	0.43	0.28	1.19	0.05	0.20	2.08	13.0	5.6	25.2
	3	14.44	1.730	.00070	.0340	.0120	.0028	4.37	0.31	0.29	0.92	0.11	0.15	1.60	10.0	6.5	25.1
Catalpa speciosa (catalpa)	1	8.15	0.380	.00180	.0330	.0080	.0034	1.17	0.48	0.58	2.47	0.16	0.23	3.68	23.0	4.7	13.2
	2	7.30	0.840	.00210	.0560	.0100	.0028	1.85	0.51	0.31	1.45	0.12	0.22	2.47	15.4	4.6	15.1
	3	9.62	0.860	.00190	.0680	.0130	.0050	2.26	0.34	0.30	1.31	0.10	0.48	1.92	12.0	5.0	15.5
Prunus serotina (cherry, wild black)	1	6.62	0.100	.00050	.0210	.0170	.0031	1.29	0.37	0.47	1.31	0.14	0.21	3.55	22.2	5.3	11.8
	2	6.45	0.180	.00060	.0210	.0200	.0072	2.37	0.55	0.14	1.14	0.10	0.06	2.63	16.4	8.8	11.9
	3	8.04	0.360	.00080	.0290	.0230	.0027	2.20	0.53	0.35	1.09	0.07	0.12	2.24	14.0	9.9	12.3
Magnolia macrophylla (cucumber tree)	1	8.16	0.160	.00080	.0150	.0030	.0059	0.90	0.30	0.48	3.30	0.19	0.29	4.31	26.9	4.6	20.0
	2	7.01	0.270	.00080	.0240	.0220	.0062	2.14	0.41	0.20	1.25	0.03	0.02	2.04	12.8	6.2	23.3
	3	9.45	1.010	.00060	.0230	.0290	.0019	2.38	0.33	0.18	1.33	0.09	0.20	1.74	10.9	8.0	19.0
Cornus florida (dogwood)	1	7.19	0.210	.00090	.0280	.0040	.0003	2.71	0.47	0.32	1.13	0.14	0.38	2.42	15.1	4.8	11.5
	2	8.91	0.560	.00070	.0380	.0030	.0014	3.58	0.27	0.20	0.51	0.08	0.59	1.54	9.6	9.1	11.0
	3	12.96	0.610	.00070	.0240	.0050	.0028	4.21	0.51	0.18	0.37	0.12	0.70	1.37	8.6	4.8	10.2
Ulmus americana (elm)	1	9.40	1.810	.00160	.0520	.0080	.0010	1.40	0.41	0.59	2.03	0.13	0.35	5.03	31.4	3.5	11.0
	2	10.36	3.270	.00090	.0810	.0110	.0022	2.42	0.57	0.14	0.92	0.20	0.02	2.20	13.8	6.0	16.6
	3	10.99	3.660	.00070	.0680	.0130	.0020	2.45	0.53	0.13	0.59	0.11	0.07	2.13	13.3	7.7	14.9
Liquidambar styraciflua (sweet gum)	1	4.83	0.240	.00150	.0210	.0310	.0002	0.79	0.49	0.37	1.62	0.07	0.26	3.02	18.9	4.8	10.0
	2	5.55	1.030	.00070	.0740	.0510	.0034	1.55	0.36	0.17	0.71	0.18	0.02	2.63	16.4	2.9	14.8
	3	8.24	1.320	.00090	.0200	.0700	.0027	1.97	0.43	0.29	0.60	0.07	0.01	1.51	9.4	3.5	15.9
Celtis occidentalis (hackberry)	1	9.15	1.370	.00080	.0610	.0100	.0029	2.53	0.38	0.44	2.27	0.11	0.32	4.28	26.8	4.2	18.0
	2	10.95	5.360	.00080	.0730	.0170	.0006	2.60	0.52	0.16	1.73	0.14	0.12	2.83	17.7	4.3	15.2
	3	20.08	5.410	.00050	.0350	.0130	.0014	5.81	0.61	0.23	1.21	0.12	0.23	2.53	15.8	5.3	15.3
Crataegus polita (haw, red)	1	9.72	0.790	.00160	.0580	.0130	.0034	3.82	0.44	0.20	1.84	0.12	0.18	4.56	28.5	4.3	18.3
	2	8.84	0.510	.00100	.0600	.0130	.0018	3.50	0.50	0.15	1.18	0.10	0.21	2.90	18.1	7.1	14.1
	3	11.98	0.570	.00110	.0530	.0150	.0030	3.76	0.44	0.14	0.92	0.09	0.03	1.70	10.7	7.0	13.28

Celtis occidentalis (hackberry)	1	9.15	1.370	.0080	.0610	.0100	.0029	2.53	0.38	0.44	2.27	0.11	0.22	4.28	26.8	9.4	3.5	15.9
	2	10.95	5.360	.0080	.0730	.0170	.0066	7.69	0.52	0.16	1.74	0.14	0.17	2.93	28.8	4.2		18.2
	3	20.08	8.410	.0050	.0850	.0170	.0034	2.81	0.44	0.26	1.84	0.13	0.182	4.36	13.2	4.4	16.72	
Crataegus polita (haw, red)	1	9.72	0.790	.0160	.0580	.0120	.0034	2.82	0.44	0.26	1.84	0.13	0.182	4.36	13.2	4.4	16.72	
	2	8.84	0.510	.0160	.0600	.0130	.0018	3.50	0.50	0.15	1.18	0.10	0.211	890	12.1	7.1	14.18	
	3	11.98	0.570	.0110	.0530	.0150	.0030	3.76	0.44	0.14	0.92	0.09	0.031	710	10.7	7.0	13.28	
Ilex opaca (holly)	1	5.25	0.100	.0140	.0250	.0260	.0130	0.54	0.26	0.40	1.98	0.09	0.213	490	21.8	6.4	21.4	
	2	4.04	0.150	.0070	.0200	.0400	.0150	1.07	0.44	0.09	0.91	0.02	0.281	510	9.4	4.7	35.5	
	3	4.82	0.220	.0060	.0270	.0540	.0240	0.97	0.51	0.07	0.74	0.09	0.331	080	6.8	5.2	33.5	
Tilia americana (linden)	1	8.93	0.130	.0080	.0220	.0070	.0021	1.43	0.40	0.62	2.48	0.42	0.344	72	29.5	3.4	12.3	
	2	12.26	0.580	.0100	.0430	.0150	.0042	5.10	0.77	0.27	1.54	0.29	0.122	45	15.3	5.3	16.2	
	3	19.64	0.540	.0100	.0330	.0210	.0054	6.43	0.81	0.22	1.12	0.25	Tr.	1.89	11.8	6.3	13.8	
Robinia pseudo-acacia (locust)	1	7.87	0.09	.....	*0.0190	.0040	.0007	1.48	0.30	0.56	2.06	0.28	0.395	94	37.2	3.4	10.8	
	2	9.19	0.230	.0070	.0330	.0050	.0047	3.42	0.30	0.29	1.69	0.08	0.033	96	24.8	4.0	15.3	
	3	14.14	0.280	.0070	.0330	.0050	.0050	4.54	0.41	0.20	1.21	0.06	0.023	12	19.5	4.5	14.6	
Acer saccharum (maple)	1	4.80	0.260	.0120	.0150	.0040	.0024	0.57	0.24	0.46	1.58	0.05	0.243	44	21.5	4.3	10.0	
	2	7.29	1.430	.0110	.0440	.0070	.0026	1.92	0.35	0.24	0.99	0.02	0.011	88	11.8	6.2	16.8	
	3	10.36	2.190	.0120	.0220	.0100	.0054	2.42	0.28	0.30	0.95	0.14	0.011	78	11.1	8.1	16.9	
Morus rubra (mulberry)		16.73	3.070	.0070	.0500	.0250	.0030	3.82	0.35	0.29	2.27	0.10	0.093	12	19.5	5.9	11.5	
Quercus velutina (oak, black)	1	4.29	0.090	.0090	.0280	.0490	.0038	0.91	0.30	0.15	1.24	0.09	0.222	76	17.3	3.1	25.2	
	2	5.10	0.430	.0090	.0260	.1480	.0068	1.56	0.36	0.17	1.16	0.05	0.252	59	16.2	3.2	23.6	
	3	7.65	0.240	.0070	.0250	.1870	.0066	2.17	0.31	0.20	1.00	0.09	0.042	22	13.9	4.1	22.5	
Quercus palustris (oak, pin)	normal†	5.29	n.d.	0.0038	0.0180	.0560	.0088	1.36	0.25	0.39	1.09	0.70	0.162	33	14.5	n.d.	n.d.	
	chlorotic‡	6.58	n.d.	0.0060	0.0450	.0050	.0116	1.31	0.21	0.39	1.67	0.75	0.223	36	21.0	n.d.	n.d.	
Diospyros virginiana (persimmon)	1	6.13	0.220	.0080	.0220	.0090	.0042	0.62	0.28	0.46	2.45	0.02	0.284	16	26.0	4.7	12.3	
	2	8.40	0.360	.0090	.0250	.0140	.0048	1.79	0.52	0.17	2.37	0.07	0.282	82	17.6	7.7	12.1	
	3	8.79	0.360	.0050	.0210	.0220	.0036	1.63	0.36	0.14	1.98	0.17	0.272	28	14.3	8.4	11.3	
Liriodendron tulipifera (poplar)	1	6.05	0.090	.0030	.0240	.0040	.0068	1.01	0.33	0.36	1.71	0.04	0.383	88	24.3	5.2	10.4	
	2	7.72	0.250	.0050	.0380	.0070	.0026	3.02	0.34	0.20	1.10	0.01	0.263	01	18.8	4.5	15.0	
	3	10.67	0.270	.0050	.0280	.0090	.0028	3.52	0.211	0.22	0.86	0.07	0.372	81	17.6	5.9	13.3	
Platanus occidentalis (sycamore)	1	6.69	0.390	.0070	.0210	.0120	.0009	1.36	0.30	0.46	1.61	0.41	0.373	49	21.9	2.7	18.3	
	2	6.32	0.540	.0070	.0180	.0120	.0039	1.76	0.31	0.15	1.40	0.04	0.422	24	14.0	2.7	23.6	
	3	9.00	0.720	.0040	.0260	.0150	.0042	2.19	0.30	0.16	1.25	0.10	0.082	07	12.9	3.2	21.9	
Juglans nigra (walnut)	1	7.32	0.400	.0110	.0280	.0060	.0049	1.06	0.35	0.54	2.04	0.09	0.234	57	28.6	4.3	11.0	
	2	9.89	0.580	.0110	.0780	.0110	.0026	2.68	0.47	0.32	2.45	0.16	0.052	18	13.6	4.6	17.4	
	3	13.07	0.640	.0110	.0400	.0190	.0042	3.23	0.50	0.46	1.98	0.15	0.011	74	10.9	5.1	17.4	
Cladrastis lutea (yellowwood)	1	8.48	0.230	.0100	.0180	.0030	.0011	1.85	0.22	0.42	2.44	0.09	0.253	53	22.1	4.5	20.5	
	2	9.15	0.780	.0070	.0360	.0050	.0040	3.23	0.25	0.54	1.81	0.03	0.132	66	16.6	4.1	24.6	
	3	13.27	1.900	.0120	.0250	.0070	.0036	3.68	0.29	0.78	1.88	0.09	0.112	24	14.0	4.4	24.0	

* Sample contaminated with copper.
† Sample collected near end of growing season.
‡ Sample collected from trees showing chlorosis due to excess of limestone in soil.

from the copper sulphide was boiled after the addition of 5 ml. of nitric acid to oxidize iron to the ferric condition, cooled, and transferred to a 200-ml. volumetric flask, made to the mark, mixed, and suitable aliquots taken for the determination of iron, manganese, zinc, calcium, magnesium, phosphorus, potassium, and sodium. Separate portions of each sample were weighed out for nitrogen, sulphur, fat, and crude fiber determinations. Moisture was estimated from the loss of weight on drying 10 gm. in air at 100° C. for 5 hours.

Table I contains the analyses. The different samplings are distinguished by the numbers 1, 2, and 3, indicating the order of collection.

Discussion of analyses

The average total water content of the first lot of samples was approximately 75 per cent; that of the second, approximately 60 per cent; and that of the third, approximately 55 per cent. These results demonstrate that as the growing season advanced the water content of the leaves decreased appreciably.

Excepting the holly, the percentages of ash of the three lots of leaves showed a very marked increase as the season advanced. In the third sampling the maximum percentage of ash was 26.98 (in the leaves of the hackberry) and the minimum, 7.65 (in the leaves of the black oak).

The different species of trees showed considerable variation in the percentage of silica in their leaves at different times during the growing season. The maximum percentage of silica was 5.41, in the hackberry leaves. Other species, in descending order of percentage of silica in the dry leaves, were elm, mulberry, maple, yellowwood, buckeye, and sweet gum. In the remaining 15 species the silica was less than 1.2 per cent.

Copper is a constant and rather uniform constituent in the leaves, but was found in smaller quantities than any of the other elements determined. The catalpa leaves contained the largest quantity of copper, and it remained practically constant in the leaves as the season advanced. The smallest amount of copper occurred in the leaves of the yellow poplar, which contained about one-fifth as much as the catalpa leaves. Copper does not vary much in the

different leaves analyzed, and the maximum amount is considerably less than either manganese or zinc, the latter elements showing considerable differences, both seasonal and among the different species. Rocks and soils contain relatively small amounts of copper, some of which is absorbed through the roots and deposited in the tissue of the plants. From its constant and uniform occurrence in the leaves, it is to be assumed that copper has an important function in the metabolism of trees.

The iron content of the different species of leaves shows considerable variation. It is greatest in elm, black walnut, hackberry, catalpa, and mulberry. The samples collected at mid-season contained appreciably more iron than those collected at the beginning and at the end of the growing season. Of the four metallic elements copper, manganese, zinc, and iron, the last occurs in the greatest amount in all species except sweet gum, black oak, and pin oak. In the leaves of these three species the manganese content considerably exceeds the iron content. From previous experiments with lower types of plants, it seems likely that each of these elements has important functions in the economy of the higher plants.

The leaves of different species of trees show considerable variation in the amount of manganese they contain. The pin oak, sweet gum, black oak, and elm contain relatively large amounts of manganese. The leaves of these trees have a characteristic deep green, glossy appearance during the growing season, which indicates that they contain a larger amount of chlorophyll than the leaves of other trees which have a lighter green color. This fact indicates a correlation between manganese content and chlorophyll. There is considerably more manganese than iron in the leaves of the sweet gum, pin oak, and black oak, whereas the iron content of the leaves of other species analyzed exceeds the manganese content. The leaves of these three species develop beautiful autumnal colors, and it is assumed that an excess of manganese over iron is probably a factor in the production of these colors. The manganese content of the leaves of nearly all the species increases as the growing season advances. QUARTAROLI (3) reports results which show that the manganese content of leaves increases with their age, and our results are in accord with his findings.

It has also been observed that when young pin oak and sweet gum trees are set in a soil containing considerable calcium carbonate, the leaves become chlorotic for several seasons and the trees die. Apparently these trees require an acid soil for a normal growth.

Zinc is a normal constituent of all of the different species of leaves analyzed; however, the amount contained in the different samplings shows some wide variations. The maximum percentage of zinc occurred in the holly leaves and the minimum in the leaves of the elm. Some of the leaves showed a gradual accumulation of zinc during the growing period. In a few species the young leaves contained a greater percentage of zinc than the mature leaves.

The calcium content of all the leaves except the crabapple, holly, and persimmon increased as the season advanced. The greater increase of this element in the leaves occurred during the interval between the first and second samplings. The maximum calcium content, 7.8 per cent, was found in the mature leaves of the hackberry. This is equivalent to 390 pounds of calcium carbonate per ton of the dry leaves. The minimum calcium content of any of the leaves, except holly, at the end of the growing season was found in the leaves of the persimmon. It was 1.63 per cent, which is equivalent to 81.5 pounds of calcium carbonate per ton of dry matter, a little less than one-fourth of the calcium carbonate equivalent of the hackberry leaves. YANOVSKY, NELSON, and KINGSBURY (7) have recently shown that the pits of hackberry seeds contain considerable calcium carbonate; it is unusual for calcium carbonate to occur in plants. Qualitative tests on hackberry pits from trees on the grounds of the Kentucky Agricultural Experiment Station on June 1, 1932, confirm their findings. The hackberry tree apparently assimilates a greater amount of calcium than any other of the species analyzed.

Under natural conditions it has been observed that certain species of trees are adapted to certain types of soil. In eastern Kentucky, where the soils have been derived from the disintegration of sandstones and shales and are low in calcium, the most common species of forest trees are black, white, and chestnut oaks, yellow poplar, ash, sweet gum, dogwood, wild cherry, red and yellow pine, beech, chestnut, sassafras, and persimmon; whereas in central Kentucky, where the soils have been derived from limestone formations, the

more common species include black walnut, burr oak, sycamore, sugar maple, hickory, locust, elm, hackberry, coffee bean, and ash.

The average calcium content of the leaves of seven species of trees common in central Kentucky, black walnut, sycamore, sugar maple, locust, catalpa, linden, and hackberry, is 4.1 per cent; whereas the average of seven species, black oak, sweet gum, yellow poplar, dogwood, wild cherry, persimmon, and ash, whose natural habitat is a sandstone soil, is 2.5 per cent. The latter trees were growing in a soil derived from limestone, however, and probably contained more calcium than leaves of the same species growing in a soil low in calcium, which is their normal habitat.

The magnesium content of the leaves of all species is remarkably constant throughout the growing season. In the majority of the samples there is a slight increase in the magnesium content as the season progresses. The two species which contained the greatest amount of calcium also contained more magnesium in proportion than the leaves which were low in calcium.

The phosphorus content in one-third of the samples of the young leaves ranged from 0.62 to 0.54 per cent, while in the remaining samples collected at the same time the range for phosphorus was 0.48 to 0.15 per cent. The leaves which contained the greatest amounts of phosphorus are, in the order named, linden, buckeye, ash, elm, catalpa, locust, and black walnut. The yellowwood, black oak, and crabapple leaves are abnormal with respect to phosphorus in that the last samples of the season contain more phosphorus than the first. In all the other species the young leaves contained the greatest percentage of phosphorus. The maximum amount of phosphorus in any of the young leaves was 0.62 per cent, which is equivalent to 12.4 pounds of phosphorus per ton of dry matter. The maximum phosphorus in the last sampling was 0.78 per cent, or 15.6 pounds of phosphorus per ton of dry matter. The minimum phosphorus, 0.2 per cent, occurred in the leaves of the black oak.

The sodium content of all the leaves was low and rather constant in amount. Although sodium is universally present in plant material, it has not been shown to have any necessary function in the economy of plants.

The percentage of potassium in the leaves of all the species was

greatest in the young leaves and diminished to about 50 per cent of the maximum by the end of the growing season. In a few species the maximum potassium content was twice as much as in others, but in the majority of the leaves the differences were less.

In most of the species the sulphur content was greatest in the young leaves and gradually diminished in the second and third samples. In the dogwood and catalpa, however, the third sample (the oldest leaves) contained nearly twice as much sulphur as the first sample. The persimmon leaves contained approximately the same amount of sulphur in each of the three samples. In the leaves of the linden the first sample contained a normal amount of sulphur but the last contained only a trace.

The nitrogen content of the leaves of the different species of trees affords several points of interest. The young leaves of the locust contained the greatest percentage of nitrogen of any of the species. The young leaves of the ash and the elm contained nearly as much nitrogen as the locust, although they do not belong to the family of legumes. The mature leaves of the locust contained 3.12 per cent of nitrogen, which is equivalent to 62.4 pounds of nitrogen per ton of moisture-free material. This amount is comparable with that contained in the leguminous forage crops.

The ether extract includes resins, chlorophyll, oily and fatty substances. Probably resins constitute a greater part of the ether extract than either chlorophyll or fatty material. The crabapple leaves contained considerably more ether extract than any of the other leaves. The ether extract from the crabapple leaves was a rather firm, compact solid, however, which led to the assumption that it contained considerably more resinous matter than fatty material. In most of the leaves the last samples contained a greater amount of ether extract than the first samples; however, the extract from the second sample of dogwood leaves was nearly twice as much as the extracts in either the first or third samples. In a few of the species the ether extract was less in the second samples than in either the first or third samples.

The crude fiber determinations show that the leaves can be put into three groups, low, medium, and high. The leaves that have a low crude fiber content, 15 per cent or less, include crabapple, ca-

talpa, cherry, dogwood, elm, gum, hackberry, haw, linden, locust, persimmon, and poplar. When dry, the leaves of these trees are quite brittle and they disintegrate rapidly in the soil. The leaves which have a medium crude fiber content, approximately 20 per cent, include the ash, cucumber, maple, sycamore, and walnut; and the species which have a high crude fiber content are buckeye, holly, black oak, and yellowwood. The leaves in the second and third groups would disintegrate more slowly in the soil than those in group one.

TABLE II

AVERAGE COMPOSITION OF LEAVES OF 21 SPECIES OF TREES COLLECTED AT THREE INTERVALS DURING GROWING SEASON OF 1931; RESULTS CALCULATED AS PERCENTAGES OF MOISTURE-FREE MATERIAL

	First sampling May 4–8	Second sampling August 3–6	Third sampling September 4–6	Nutrients (lb. per acre)*
Ash (crude)	7.150	8.459	11.449	458.00
Insoluble residue (SiO_2)	0.376	0.951	1.159	46.40
Copper (Cu)	0.001	0.0009	0.0009	0.04
Iron (Fe)	0.029	0.0429	0.0337	1.35
Manganese (Mn)	0.011	0.0209	0.0265	1.06
Zinc (Zn)	0.003	0.0042	0.0046	0.18
Calcium (Ca)	1.329	2.767	3.142	125.68
Magnesium (Mg)	0.353	0.422	0.404	16.16
Phosphorus (P)	0.452	0.234	0.274	10.96
Potassium (K)	1.997	1.344	1.173	46.90
Sodium (Na)	0.165	0.087	0.116	4.60
Sulphur (S)	0.281	0.187	0.170	6.80
Nitrogen (N)	3.829	2.365	1.970	78.80
Protein (N×6.25)	23.930	14.78	12.310	492.40
Ether extract	4.910	5.57	6.210	248.30
Crude fiber	13.690	18.12	16.990	679.80

* DAY (1) assumes an annual leaf-fall in forests of more than 2 tons per acre.

There seems to be a very general impression among plant physiologists and botanists that toward the end of the growing season there is a gradual transfer of plant nutrients from the leaves back to the branches, trunk, and roots of trees. The averages of the different constituents in the first, second, and third samplings were computed to ascertain whether there were any trends among the different constituents determined confirming this very general idea. Table II contains the averages of the different constituents for the three different samplings. The results are percentages of the moisture-free material.

In table II it is to be observed that the ash, insoluble residue, manganese, zinc, calcium, and ether extract showed a decided increase in the leaves as the season advanced, whereas potassium, sulphur, and nitrogen showed a gradual decrease during the growing season. Copper remained practically unchanged; iron, magnesium, and crude fiber attained a maximum in the second sampling and declined slightly in the third. Phosphorus declined nearly 50 per cent between the first and second samplings and regained appreciably in the third sampling. Nitrogen, potassium, and sulphur are the only elements that showed a slight diminution in the interval between the second and third samplings. It is assumed that this diminution is only apparent and is due principally to the formation of cellulose and other compounds which dilute the potassium and nitrogen content as the season advances. These results do not afford any convincing evidence that there is a migration of plant nutrients from the leaves to the branches of trees toward the latter part of the growing season.

It is reported that in some European countries the leaves of certain species of trees are gathered and used as a substitute for hay. In this country, however, no such practice is common. In the spring, when the leaves of trees are young and tender and grass scarce, cattle will feed on forest leaves, but other livestock apparently do not relish this kind of forage under ordinary circumstances.

Summary

1. The leaves of 23 species of deciduous trees were analyzed at three intervals during the growing season. The results show that the dry matter of young leaves contained the largest percentages of phosphorus, potassium, and nitrogen, whereas the mature leaves collected at the end of the growing season contained the largest percentages of ash, silica, and calcium. Copper, manganese, and zinc were normal constituents in all of the samples. In three of the species, sweet gum, black oak, and pin oak, the manganese content was in excess of the iron content, which is an unusual relationship. The leaves of these trees develop beautiful colors during the late autumn season, and the unusual relationship of iron and manganese may be a factor in the production of these autumnal shades.

2. The results do not afford material evidence that mineral nutrients migrate from the leaves to the branches toward the end of the growing season.

3. A mixed forest probably would add as much plant nutrient to the surface soil in an annual crop of leaves as is removed by the average forage crop.

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CYTOLOGY OF ANCHUSA AND ITS RELATION TO THE TAXONOMY OF THE GENUS

STANLEY G. SMITH

(WITH SEVEN FIGURES)

Introduction

The great diversity of form to be found within the genus *Anchusa*, together with the clear-cut differences exhibited between the species, suggested that the cytology of the genus might be interesting. After a preliminary study of several of the common species, it was so surprising to find in *A. myosotidiflora* Lehm. a chromosome complex differing entirely from any previously seen, that Dr. O. STAPF, of the Royal Botanic Gardens, Kew, England, was consulted as to the validity of its classification. It was gratifying, therefore, to learn that recently, agreeing with JOHNSTON (9), STAPF (15) had published a description of the plant, treating it as a member of the restored genus *Brunnera*.

According to JOHNSTON, this species was originally described by MARSCHALL (13) as *Myosotis macrophylla;* then by LEHMANN (11) as *Anchusa myosotidiflora*. STEVEN (16) separated it from the genus *Anchusa* and assigned it to the closely related and newly described genus *Brunnera*, calling it *B. myosotidiflora*. This splitting of the genus, however, was not accepted until the publication of JOHNSTON'S study of the Boraginaceae (9). He came to the conclusion that *Anchusa myosotidiflora*, with its variety *grandiflora*, and *A. neglecta*, plants indigenous to Siberia and the eastern Mediterranean region, exhibited sufficient differences to warrant their treatment as generically distinct from *Anchusa*. Following the law of priority for the specific epithet, the name of *Anchusa myosotidiflora* automatically became *Brunnera macrophylla* (Marschall) Johnston.

Among the material examined before JOHNSTON'S paper was received, *Anchusa sempervirens* L., a species barely justifying its inclusion in the genus on account of its distinct morphology, was found to possess a complement of chromosomes so markedly dis-

similar in size and number from all others up to this time examined that, on the analogy of *Brunnera macrophylla*, it also seemed to merit its early recognition as the distinct genus *Caryolopha* Fisch. and Traut (**5**).

A tabular comparison of JOHNSTON'S arrangement with that of GÜRKE (**6**) follows. In the cytological observations, the nomenclature used throughout is that given by the latter investigator. The species are discussed in the order in which they appear here.

ANCHUSEAE

GÜRKE'S CLASSIFICATION			JOHNSTON'S CLASSIFICATION	
Anchusa	Section I, Buglossum	A. barrelieri	Anchusa	
		A. ochroleuca		
		A. italica		
	Section II, Buglossoides			
	Section III, Euanchusa	A. hybrida		
		A. officinalis		
	Section IV, Caryolopha	A. sempervirens	Caryolopha	
	Section V, Myosotoides	A. myosotidiflora	Brunnera macrophylla	

Material and methods

A list of the plants examined is as follows.[1] They were kindly supplied by the directors of the various institutions mentioned.

Anchusa sempervirens.....Royal Horticultural Society, Cambridge Botanical Gardens, John Innes Horticultural Institution
A. myosotidiflora.....Royal Horticultural Society
A. barrelieri.....Chelsea Physic Gardens
A. ochroleuca (seeds).....Valencia
A. capensis.....John Innes Horticultural Institution
A. hybrida.....John Innes Horticultural Institution
A. italica.....John Innes Horticultural Institution
A. italica var. Pride of Dover.....John Innes Horticultural Institution
A. italica var. Dropmore.....John Innes Horticultural Institution
A. italica var. Opal.....John Innes Horticultural Institution
A. officinalis.....Royal Botanic Gardens

[1] Illustrations of these plants may be seen in the following volumes of CURTIS'S Botanical Magazine: *A. barrelieri* 49 tab. 2349, *A. capensis* 43 tab. 1822, *A. italica* 48 tab. 2197, *A. ochroleuca* 39 tab. 1608, *B. macrophylla* 151 tab. 9110. *C. sempervirens* is shown by GUSULEAC in Repert. Spec. Nov. Fedde **29**:42–47. 1931.

Plants were grown in pots and root tips were fixed in La Cour's (10) 2BE fixative. Material was cut at 10, 14, and 20 μ, the thickness varying with the size and number of chromosomes, and stained by a chromic acid modification of Newton's gentian violet method (La Cour 10).

Somatic chromosomes

In Section I, Buglossum, six plants (*A. barrelieri*, *A. ochroleuca*, *A. italica* and three of its garden varieties) were examined for somatic chromosome number and form.

Anchusa barrelieri (fig. 1) was found to be diploid with $2n = 16$ chromosomes, of which one type with a subterminal attachment constriction, another with a slight almost terminal secondary constriction, and the smallest of the complement with a median point of attachment, were present in duplicate only. The remaining types were difficult to determine with any degree of certainty, but, taking other cells into consideration, the whole complement seemed to be:

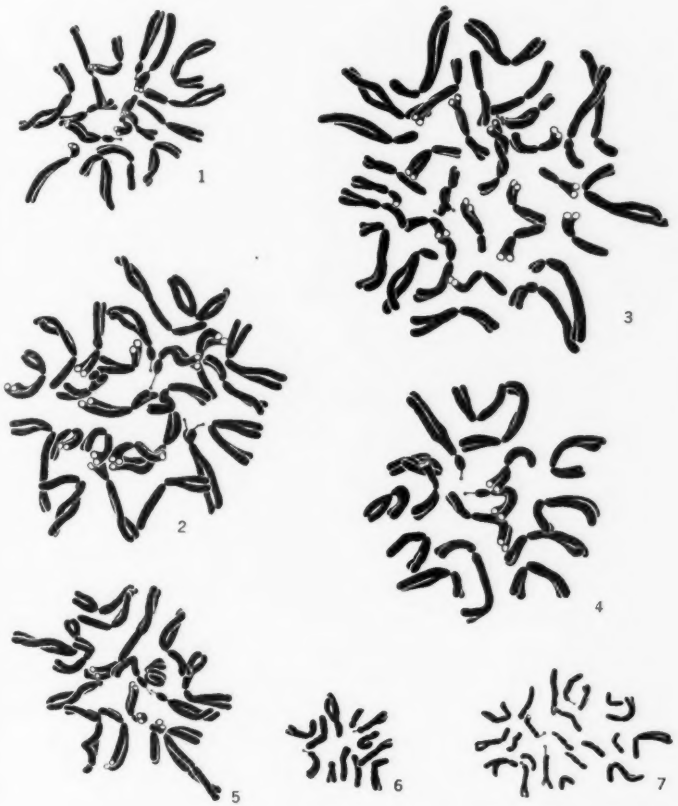
1 pair with a subterminal constriction and a trabant on the shorter segment;
2 pairs with median constrictions;
5 pairs with submedian constrictions.

Anchusa ochroleuca seedling (fig. 2) arose from seeds received from Valencia and gave a somatic count of 24 chromosomes. This plant has a complement of chromosomes in triplicate, the most obvious of which are the three subterminally constricted chromosomes bearing trabants. The others without doubt are present also in triplicate, although this is not apparent in the figures, owing to their large size and contorted shape and the limitations of two-dimensional representation. The arrangement of the chromosomes with regard to their points of attachment was found to be:

3 chromosomes with subterminal constrictions and trabants on the minor segments;
6 chromosomes with median constrictions;
15 chromosomes with submedian constrictions, one of which had a slightly secondary constriction.

This triplicate constitution indicates that the seedling is an autotriploid derived from the fertilization of an abnormal diploid ovule

by a normal haploid gamete, or vice versa. The former seems to be the more probable in the light of research on tetraploid *Datura* (BLAKESLEE, BELLING, and FARNHAM 3).



FIGS. 1–7.*—Somatic metaphase plates from root-tip cells of seven species of the Anchuseae: fig. 1, *Anchusa barrelieri* (2n=16); fig. 2, *A. ochroleuca* (2n=24); fig. 3, *A. italica* var. Pride of Dover (2n=32); fig. 4, *A. officinalis* (2n=16); fig. 5, *A. hybrida* (2n=16); fig. 6, *Brunnera macrophylla* (syn. *A. myosotidiflora*) (2n=12); fig. 7, *Caryolopha sempervirens* (syn. *A. sempervirens*) (2n=22). ×4200.

*All drawings made with the aid of a camera lucida, a Zeiss 2 mm. objective, N.A. 1.3, 30 × ocular.

Unfortunately this plant and its sibs were inadvertently destroyed before the cytological material was examined, and there is therefore no way of determining whether or not the triploid condition was exceptional. Nevertheless, it seems particularly significant that the one plant chosen as a typical healthy representative of a batch of seedlings should, throughout all the root tips, exhibit triploidy with which is usually associated a certain degree of dissimilarity from the true diploid. It is hardly possible that this plant was a segregate from a triploid, as the chance of two gametes combining to restore a triply balanced set of chromosomes is extremely rare (BELLING **2**), even in the first generation from a newly arisen triploid.

Anchusa italica and its varieties Pride of Dover, Dropmore, and Opal have $2n = 32$ chromosomes. In Pride of Dover (fig. 3) the following types of chromosomes were noted:

2 pairs with subterminal constrictions, the smaller pair bearing trabants on the major arms;
3 pairs with median constrictions;
11 pairs with submedian constrictions, including one with a secondary constriction situated on the minor segment close to the point of attachment. The longest pair of chromosomes, which were in this class, had a slight secondary constriction about half-way along the major arm.

In all these plants only two chromosomes of each distinguishable shape were present, as in *Sorghum halepense* (HUSKINS and SMITH **8**), which very strongly suggests that they are of an allotetraploid origin similar to that of *Primula kewensis* (NEWTON and PELLEW **14**).

In the variety Opal there was a fragmented chromosome. This was probably additional to the normal tetraploid complement, but difficulty was experienced in obtaining a preparation sufficiently well fixed to show the entire set.

Of the species included in Section III, Euanchusa, three (*A. officinalis*, *A. hybrida*, and *A. capensis*) were examined. Two are illustrated here, and the third, *A. capensis*, had an apparently similar complement.

Anchusa officinalis (fig. 4) was a normal diploid with 16 somatic chromosomes, which could be classified as:

1 pair with a subterminal constriction and a trabant on the minor arm;
3 pairs with median constrictions, one of which had a subordinate, almost terminal, constriction which was less obvious and only occasionally observed;
4 pairs with submedian constrictions, including one pair of much greater length than the others.

Anchusa hybrida (fig. 5) possessed a complement of 16 chromosomes strikingly similar to *A. officinalis* and *A. capensis*. The eight different types could be arranged as:

1 pair with a subterminal constriction and a trabant[2] which was again situated on the minor arm;
3 pairs with median constrictions;
4 pairs with submedian constrictions. The longest pair possessed a sharp secondary constriction about half-way along the major arm, which divided the chromosome into three almost equal segments. (An indication of the same condition is to be observed in a comparable chromosome of the *A. ochroleuca* seedling illustrated in figure 2.)

Section IV, Caryolopha.—This is a monospecific section, having *A. sempervirens* (*Caryolopha sempervirens*) as its sole representative. Somatic counts gave a diploid complement of 22 chromosomes (fig. 6), all of which were decidedly smaller than those of the previous sections. Some of the morphological types seemed to be present in quadruplicate, although, as is obvious from the illustration, it would be inadvisable to state this definitely. These chromosomes also could be arranged with regard to their points of attachment into three classes:

[2] Other cells were seen in the same root tip in which each chromosome of this pair bore a trabant on the shorter segment, but the trabants were so small that difficulty was encountered in finding them in every cell, even though their position was known. These elements have therefore been included in the drawing, although they were not observed in this particular cell.

2 pairs with trabants and subterminal constrictions, one pair of which had a secondary constriction at the end distal to the trabant;
3 small pairs with median constrictions;
6 pairs with submedian constrictions, including one very large pair.

Anchusa myosotidiflora (*Brunnera macrophylla*), the only species examined in GÜRKE's Section V, Myosotoides, was found to have $2n = 12$ chromosomes (fig. 7), all of which had approximately the same mass as those of the preceding section. The members of this complement were found to fall into the following classes:

2 pairs having subterminal constrictions, the larger of which was in accordance with the general rule in that it possessed a trabant on the minor arm;
1 pair with a median constriction;
3 pairs with submedian constrictions.

Discussion

Table I compares the two systems of classification diagrammatically in relation to the cytological results. It is particularly

TABLE I

GÜRKE'S CLASSIFICATION	2n CHROMOSOME NUMBER	CHROMOSOME TYPES CLASSIFIED ACCORDING TO POSITION OF POINT OF ATTACHMENT			CHROMOSOME LENGTH (μ)		JOHNSTON'S CLASSIFICATION
		SUBTERMINAL	MEDIAN	SUBMEDIAN	SHORTEST	LONGEST	
Anchusa barrelieri	16	2	4	10	4.2	6.1	Anchusa barrelieri
A. ochroleuca seedling	24	3	6	15	5.5	8.0	A. ochroleuca seedling
A. italica var. Pride of Dover	32	4	6	22	4.7	8.5	A. italica var. Pride of Dover
A. officinalis	16	2	6	8	5.9	8.5	A. officinalis
A. hybrida	16	2	6	8	5.2	7.8	A. hybrida
A. sempervirens	22	4	6	12	1.8	3.8	Caryolopha sempervirens
A. myosotidiflora	12	4	2	6	1.4	3.0	Brunnera macrophylla

noticeable that the more important changes in classification occur with plants which are macroscopically different from the type most

generally accepted by horticulturists and systematists to be *Anchusa*, and which appear to be of doubtful classification from a consideration of their microscopic differences, both in external and internal morphology.

From a consideration of its chromosomal constitution, we may safely regard *Anchusa italica* as a new and relatively constant form evolved by hybridization and subsequent doubling of the hybrid complex like *Spartina townsendii* (HUSKINS **7**) and other cases. One might therefore consider this as concrete support for JOHNSTON'S assumption that phylogenetic relationships in this group of plants are reticular rather than dendritic. The cytology also justifies JOHNSTON'S action in combining the two sections, Buglossum and Euanchusa.

On cytological grounds the segregation by JOHNSTON of both *Anchusa myosotidiflora* and *A. sempervirens* into other genera as *Brunnera macrophylla* and *Caryolopha sempervirens* is completely vindicated. They show a dissimilarity in size and basic number existing between them and the genus to which they had previously been referred. But although these obvious differences in nuclear constitution occur, there are nevertheless one or two strikingly similar chromosome forms observable in all three genera. For example, the trabanted subterminally constricted chromosome persists, with one exception (*Anchusa italica* var. Pride of Dover, fig. 3), throughout the plants examined, and, further, the longest pair of chromosomes is represented in nearly all the species with an occasional modification in the form of a secondary constriction (figs. 3, 5). In view of the increasing evidence indicating that chromosome size or structure may be governed by genes (LESLEY and FROST **12**, DARLINGTON **4**, and HUSKINS and ARMSTRONG, unpublished), it seems reasonable to assume that gene mutation may have been responsible for some of the differences in the present case.

Although we are not here concerned with specific ranking, the question might be raised whether, on the analogy of *Brunnera* and *Caryolopha*, the extreme similarity among the chromosome complexes of *A. barrelieri*, *A. capensis*, *A. hybrida*, and *A. officinalis* indicates a necessity for grouping them all into one species. But as BABCOCK (**1**) points out, specific differences are not necessarily ac-

companied by visible changes in the chromosomes; or, in other words, while generic status is usually accompanied by definite chromosome variation, specific differentiation is more often effected by an accumulation of genic differences having no visible effect on gross chromosome structure.

Summary

1. *Anchusa italica* and its varieties are shown to be allotetraploids. This is in accord with JOHNSTON's theories of reticular phylogenetic relationships in the Boraginaceae.

2. The following chromosome numbers were found: *Anchusa barrelieri* 2n = 16, *A. capensis* 2n = 16, *A. hybrida* 2n = 16, *A. officinalis* 2n = 16, *A. ochroleuca* 2n = 24, *A. italica* 2n = 32, *A. italica* vars. Pride of Dover, Dropmore, and Opal 2n = 32, *Brunnera macrophylla* 2n = 12, and *Caryolopha sempervirens* 2n = 22.

3. The origin of the triploid seedling of *Anchusa ochroleuca* is discussed.

4. The removal by JOHNSTON of *Brunnera macrophylla* from the genus *Anchusa* is substantiated by the number and character of its somatic chromosomes.

5. The similar systematic treatment of *Caryolopha sempervirens* is also supported by cytological observation.

6. JOHNSTON's combining the sections Buglossum and Euanchusa is in agreement with their cytological similarity.

This study was carried out chiefly at the John Innes Horticultural Institution, Merton, England, being initiated at the suggestion of the Director, Sir A. D. HALL. The author wishes to express his indebtedness to Dr. C. LEONARD HUSKINS and Dr. EDGAR ANDERSON for their valuable criticisms of the manuscript.

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FASCIATION IN RED PINE

RAYMOND KIENHOLZ

(WITH TWELVE FIGURES)

Introduction

The peculiar flattening, or fasciation, of normally cylindrical shoots is frequently encountered in herbaceous plants. Notable in this respect is *Celosia cristata*, whose fastigiate character is inheritable. Woody plants seem to exhibit fasciation less frequently than herbaceous plants, and conifers less frequently than broad-leaved trees. Among conifers, spruce seems particularly subject to fasciation and pines less so. Early in the study of the phenomenon of fasciation it was believed that the thin, flat twigs were caused by the growing together of several twigs, but this idea is no longer held. Some internal physiological disturbance causes the growing *point* to become a growing *line*, resulting in the production of flattened twigs. The cause and nature of this physiological disturbance are not known. Pressure experimentally applied to the growing tip has not produced fasciation. TANNER (7) believes fasciation to be associated with over-nutrition.

A considerable amount of literature is devoted to the description of fasciation, but the great majority of this work, particularly as it applies to conifers, is European. An easily procurable, general account of fasciation may be found in MASTER'S (4) well known work on vegetable teratology. SCHENCK (5) compiled much information dealing with fasciation in conifers and published several fine illustrations showing the flattening, bending, torsion, and abundant production of twigs characteristic of this phenomenon. TANNER has more recently described and figured fasciation in forest trees. In this country fasciation in conifers has apparently seldom been observed. HUBERT (3) figured a fasciated twig of larch, and BALDWIN (1) described a fasciated leader of Scotch pine which showed the characteristic flattening (22 mm. wide by 10 mm. thick), the division into two, the bending due to differential growth, and the large number of

buds at the tip. Fasciation has also been observed in Douglas fir, western hemlock, and Sitka spruce.

Even in Europe, however, fasciation occurring over several successive years seems to be very rare, since SCHENCK (**6**) gave as his reason for adding to the "already over-rich literature" the fact that so far only a few cases of fasciation extending over several successive years had been described in an exact morphological manner. He described (**5**) fasciation extending over successive years in *Araucaria cunninghamii*. He also cites DE VRIES (**2**), who described fasciation affecting six successive years of growth of a specimen of *Picea*. TANNER cites examples of fasciation extending over several years.

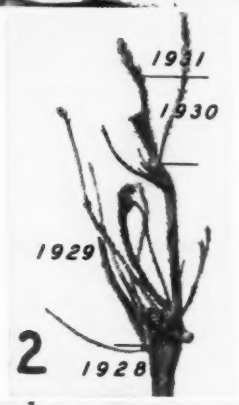
This phase of the subject is of particular interest, since little information is available concerning the length of life of fasciated branches after the phenomenon first appears. Likewise, whether fasciation continues to manifest itself each year after its first appearance is unknown.

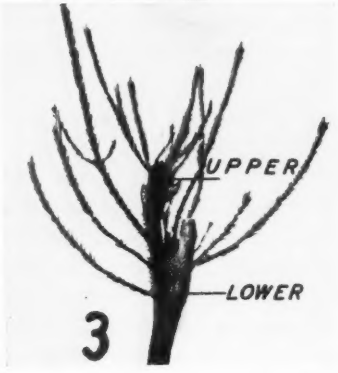
Observations

The fasciation here described occurred in the leader and upper laterals of a specimen of *Pinus resinosa* during five years (1927 to 1931) of its growth. This pine was growing in an open plantation spaced 8 by 8 feet on a gently sloping southwest exposure, located in the Yale Demonstration and Research Forest near Keene in southern New Hampshire. The soil is a fine sandy loam with a fine gravelly subsoil, moderately well watered. The crowns of the trees in the plantation were just beginning to touch and in a few cases the lower limbs had begun to die. The fasciated pine was 18 years old and about 6 meters high.

In October 1931, the upper part of the tree was cut off, photographed, and measured. The upper fasciated portion of the tree (fig. 1) was of a very dense, bushy appearance. The first distinct appearance of fasciation occurred in the 1928 leader, but even below this the number of lateral branches per whorl was greater than that of the surrounding normal trees. Table I gives the number of laterals per whorl for the fasciated tree in comparison with the average of five normal trees growing nearby.

A section through the upper part of the 1927 leader (fig. 8) showed the first annual ring to be distinctly flattened (13 by 5.5 mm.),





FIGS. 1–5.—Fig. 1, general view of fasciated growth, with the 1928 laterals removed, the whole about 1.7 meters tall. Note bushy, leafy appearance of fasciated growth and the overtopped 1928 leader to right. (Same as fig. 6.) Fig. 2, overtopped 1928 leader, with needles removed, showing flattening and excessive production of twigs. (Same as

being over twice as broad as it was thick. Later, annual growths tended to round out the stem, and the diameters of this section in 1931 were 67 and 63 mm.

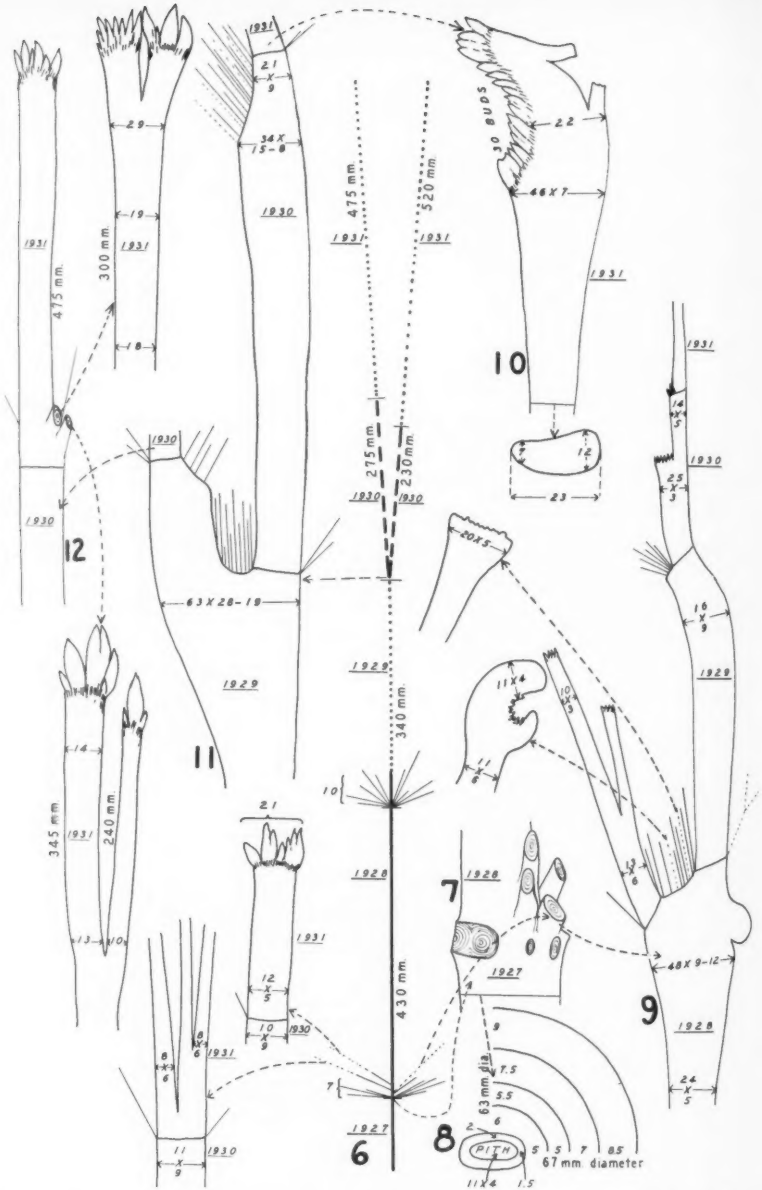
TABLE I

	1927	1926	1925	1924	1923	1922	1921	1920
Fasciated	8.0	8.0	4.0	6.0	5.0	5.0	4.0	3.0
Normal	4.8	3.6	3.6	3.2	3.4	3.0	2.8	2.4

The 1928 leader as it developed became distinctly fasciated, producing a greatly flattened tip (48 mm. wide by 9 mm. thick on one edge and 12 mm. thick on the other edge) bearing many buds (figs. 2, 9). The next year many of these buds grew out into twigs, some of which showed fasciation, others not. The buds at either edge of the broad flat tip were the stronger and produced larger, more vigorous, usually fasciated twigs that grew for several years. The buds in between produced smaller twigs which were more apt to be normal. The fasciated twig to the right produced six small twigs in 1930 and none in 1931; and the buds for 1932 were so small and dry as to appear dead. The whole leader was so completely overtopped and so weakened in its growth that it was probably only a matter of a few years before it would have died.

A normal-appearing 1928 lateral bent up to take the place of the fasciated 1928 leader. This new leader, however, exhibited extensive fasciation in 1929 and in later years (figs. 3, 11). Its greater vigor was shown by its length growth during the years 1929, 1930, and 1931. Here again the broad, flattened tip of the 1929 growth (63 mm. wide by 28–19 mm. thick) produced two larger vigorous shoots both of which developed strongly, causing a forking of the

fig. 9.) Fig. 3, part of the new 1928 leader, with needles removed, showing lower mass of twigs produced in 1930 and upper mass produced in 1931. (Same as fig. 11.) Fig. 4, detailed view of example of extreme fasciation in 1931 twig (needles removed). Edges of twig are thicker than middle, which is slightly dished. Largest buds are borne on the edges (*A* and *B*). More rapid growth on right-hand edge is shown by greater distance between the leaf scars and by position of the bud-bearing surface. Stresses set up by unequal growth resulted in splitting away of the tissue at *C*. (Same as fig. 10.) Fig. 5, moderately fasciated twig (1931 growth) showing forking at tip, and excessive bud and needle production. (Same as twig shown at right and above in fig. 12.)



FIGS. 6–12.—Diagrammatic and semidiagrammatic representation of fasciated growth, not drawn to scale; all dimensions in mm. Fig. 6, diagram of entire fasciated growth showing years and lengths. Fasciated growth indicated by dotted and dashed

tree. Between these two shoots ten small twigs were produced. These are shown in fig. 3 as the lower mass of twigs. The branch to the right continued flattened in 1930 (34 mm. wide by 15–18 mm. thick) and produced 16 small twigs in 1931, some fasciated, others not (upper twig mass in figs. 3 and 11), and one large vigorous twig which produced the most extreme example of flattening found on the tree (figs. 4, 10). The branch to the left produced a vigorous fasciation (fig. 12).

The extreme flattening of the 1931 twig on the right is shown by its dimensions of 46 mm. broad and an average of 7 mm. thick (fig. 4). It is slightly thicker at either edge and somewhat dished in the middle. The needles borne on this central dished portion are shorter than those borne along the two edges. They are nearly all normal in number, that is, two per fascicle. The total number of needles produced on a fasciated twig seems to be greater than on a normal twig, which, together with their greater crowding, caused the fasciated twig to assume a very bushy appearance. The fact that the two edges of the twig are thicker than the middle may account for the production of more vigorous buds at those points.

Many of the fasciated twigs are characterized by a more rapid growth along one edge than along the other, causing the bud-bearing tip to assume a shape such as that shown in figure 4. Rapid and unequal growth accompanied by bending caused the cracking and splitting away shown at *C* in figure 4. This condition is frequently seen in cases of extreme fasciation in herbaceous plants.

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lines, normal growth by solid lines Note laterals at base of 1928 and 1929 growth of leader. Arrows lead to portions shown in detail. Fig. 7, base of new 1928 leader showing origin of several laterals at different levels and the overtopped 1928 leader. Fig. 8, section through upper part of 1927 leader showing greatly flattened pith and first-year wood. Later growths tended to round up the section somewhat. Diameters at end of 1931 were 63 and 67 mm. Fig. 9, overtopped 1928 leader showing unequal forking due to production of most vigorous twigs at the two ends of growing *line*. Twigs produced between (10 in number) are not drawn to scale, but fasciation is shown by dotted lines, normal twigs by solid lines. Fig. 10, extreme fasciation (1931 twig) showing dimensions, buds, and splitting at upper edge. Section through base of this growth shows width of 23 mm., and thickness at one edge of 7 mm. and at other edge of 12 mm. Fig. 11, new 1928 leader showing forking at beginning of 1930 growth with the two edges producing strong twigs. Numerous (10) smaller twigs produced between and also at base of 1931 growth (16 twigs). Fig. 12, twigs (1931) showing moderate fasciation.

Discussion

Apparently the effects of this physiological disturbance within the tree became more severe from the time of its first pronounced appearance (1928) to the time when the specimens were collected (October, 1931). Although the 1927 leader appeared normal at the time of collection, a section through the upper portion showed its early growth to be flattened (fig. 8). The greater number of laterals per whorl (1920–1927) is probably indicative of a still earlier tendency toward fasciation. The laterals of the 1928 and 1929 whorls showed few signs of fasciation, while the 1930 and 1931 growths were very severely fasciated, with the 1931 growth showing the greatest amount and the most extreme examples of fasciation. TANNER (**7**) shows that normal cylindrical twigs may give rise to fasciated twigs, and on the other hand fasciated twigs may give rise to normal twigs. This is borne out in the present study.

The greatly weakened growth of the 1928 leader in 1930 and 1931 (fig. 9) indicates what effect the disease may have. Very likely the two 1931 leaders would also have become greatly weakened in their growth had they been allowed to continue growing. Continuation of such stunting effects, accompanied by extreme production of twigs and forking, would materially lessen the value of the tree for the production of lumber.

It will be of considerable physiological interest to observe whether one of the apparently normal laterals in the whorl, just below where the top of the tree was cut off when the specimens were gathered, will grow erect and exhibit fasciation in its new growth.

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COMPOSITION OF THE BEANS OF PARKINSONIA ACULEATA

ROBERT A. GREENE

Introduction

Parkinsonia aculeata, known also as the "Jerusalem thorn," and more commonly in the southwest as "palo verde" or "bagote," is common to all tropical countries. It is found in abundance in the southwest, where the tree is often cultivated for ornamental purposes, but also grows wild.

This species is very hardy and has a low water requirement. It is found on the bajados, canyons, and especially in the foothills near Tucson, where the average annual rainfall is about 12 inches. This plant shows a definite physiological response to environment. The leaves are composed of one to several long rachises and numerous minute leaflets, this adaptation materially reducing the transpiration losses. The chlorophyll and pigments associated with photosynthesis are found chiefly in the branches and stems, giving the tree a green appearance; hence the colloquial name "palo verde." Consequently practically all photosynthetic processes are carried on in the bark of the tree, rather than the leaves.

Parkinsonia aculeata is a legume, producing a considerable quantity of oblong seeds, contained in narrow pods 2–4 inches long. The seeds make up approximately 70 per cent of the weight of the whole bean. In Arizona these mature in the late summer, usually about the months of July and August.

In spite of the relative abundance of this plant, it has but little use at present, and apparently no studies of the chemical composition of the seed have ever been made. Apparently the only mention of the use of these seeds for feed by animals was made by Dr. R. H. FORBES of Tucson, who saw African monkeys strip the pods from the trees, break them open and eat the seeds. Usually the monkeys eat the seeds before they are fully ripe since the seed coat is not so hard at the time. On the range, live stock eat some of the small

tender twigs. FORBES and SKINNER (4) state: "palo verde twigs are an instance of browse forage upon which animals are so largely dependent in a semi arid country where grass is short."

Dr. J. J. THORNBER informed the writer that sometimes the Indians use the seeds as food, either cooked or raw. Since the seeds of leguminous plants contain considerable quantities of protein, this study was commenced in order to determine what possible food value the seeds might have.

Experimentation

The seeds were gathered on the campus of the University of Arizona, and since they were collected from a considerable number of trees, the sample should be representative.

TABLE I

FEEDING ANALYSIS (AIR-DRY BASIS)

	PERCENTAGE		
	PODS ONLY	WHOLE BEAN	SEEDS ONLY
Moisture	5.04	5.50	5.70
Ash	3.85	3.99	4.14
Protein	5.75	16.37	25.12
Fat	0.09	1.11	1.83
Crude fiber	50.38	27.94	19.84
Nitrogen-free extract	34.89	45.09	43.37

The seeds are contained in a narrow pod averaging about 3 inches in length. A quantity of the pods were threshed by placing them in a sack, beating with a small club, and then separating the pods from the seeds by winnowing. Both the pods and seeds were ground separately, and some of the "beans" were ground whole, without separating the pods and seeds.

The seeds are somewhat oblong in shape, have an extremely hard seed coat, and are very difficult to grind. On account of this hardness they must be soaked for four or five hours in concentrated sulphuric acid to promote germination. They may be exposed to the acid for a longer period of time without causing injury.

The pods, seeds, and the whole beans were finely ground, and the usual feeding analysis was made, using the methods of the Association of Official Agricultural Chemists (1). In order to secure

some idea of the carbohydrate composition of the pods, a proximate analysis was made, according to the methods of WAKSMAN and STEVENS (7). These results are given in tables I and II.

TABLE II

PROXIMATE ANALYSIS OF PODS
(MOISTURE-FREE BASIS)

	PERCENTAGE
Ether-soluble fraction	0.09
Cold water-soluble organic matter	6.17
Hot water-soluble organic matter	2.27
Alcohol-soluble fraction	1.49
Hemicelluloses	30.97
Celluloses	22.81
Lignins	19.56
Crude protein	4.92
Ash	4.05
Total	92.33

The seeds were treated with various peptizing agents, in order to determine the classes of proteins present. The method was similar to that employed by JOHNSON (6) in his study of tubercule bacilli. The results appear in table III.

TABLE III

DISTRIBUTION OF NITROGEN IN SEEDS

	PERCENTAGE TOTAL N
Albumin (water-soluble)	18.41
Globulin (soluble in 10% NaCl)	6.47
Glutelins (soluble in 0.2% NaOH)	13.18
Prolamines (soluble in 70% C_2H_5OH)	5.72
Other proteins (by difference)	56.22

Discussion

Table I shows that the pods contain large amounts of crude fiber and nitrogen-free extract. A proximate analysis made by the method of WAKSMAN and STEVENS (7) showed that hemicelluloses, celluloses, and lignins make up about 73 per cent of the dry weight of the pods. Further tests indicated that the pods contain considerable quantities of pentosans. Possibly the pods might be used for the manufacture of paper pulp or for the preparation of furfural from the pentosans.

Since the market is at present flooded with readily available materials for these purposes, there seems to be no commercial possibilities for utilizing the pods.

The composition of the whole bean is similar to that of a good grade alfalfa hay (**2, 5**). The crude fiber content of the two are practically the same, which indicates that each should have the same relative digestibility. There is also a similarity in composition between the whole bean of this plant and those of mesquite (**3**).

The seeds contain a considerable amount of protein but only a small percentage of fat or oil. This quantity is entirely too small to have any commercial value. The amount of crude fiber is somewhat less than that of the whole bean, but in this case the determination is not a true index of digestibility, since the seeds had been ground. It is extremely doubtful whether an animal could crush the seeds, although if they were ground they could be used as an emergency forage. The fact that the seeds are sometimes used as human and animal food is evidence that cyanogenetic glucosides or other toxic substances are not present.

Table III shows that an albumin and glutelin are probably the principal proteins present. The results for globulins appear low, since they are usually the principal proteins present in seeds. In this case, however, the globulins were extracted by 10 per cent sodium chloride, which is usually employed for such purposes. The studies of JONES, JOHNS and coworkers at the United States Department of Agriculture show that 10 per cent sodium chloride is not always the best peptizing agent for globulins. It is probable that lower concentration might have peptized a large percentage of the globulin. It is planned to extend this study by isolating the proteins and to determine the various amino acids present.

Summary

1. The pods, whole beans, and seeds of *Parkinsonia aculeata* have been analyzed. Hemicelluloses, celluloses, and lignins compose approximately 73 per cent of the pods.

2. The composition of the whole bean is similar to that of alfalfa hay or mesquite beans.

3. The principal proteins of the seed are albumin and glutelin.

4. If proper means for grinding these beans or seeds are available, they should provide a good emergency forage.

The writer acknowledges his indebtedness to Dr. E. L. ANDERSON who collected the beans; and to Dr. J. J. THORNBER who identified the species and offered many valuable suggestions.

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RELATION OF LENTICELS AND SURFACE AREA TO RESPIRATION IN THE POTATO TUBER

W. H. MICHAELS

That the permeability of the periderm of the potato tuber is a limiting factor in the exchange of gases between the tissues of the tuber and the surrounding air has been shown by a number of workers, of whom SMITH (3) is probably the most recent. So far as the writer is aware, no one has attempted to determine the relationship existing between the lenticels and the gaseous exchange of the tuber. The present paper reports such a study.

The work reported here was done at South Dakota State College during the winter of 1924–25, with potatoes which had been grown on the College farm the previous season. Clean, smooth, apparently disease-free tubers of the Irish cobbler, Early Ohio, and Green Mountain varieties were used.

The respiration rate was determined by the continuous aeration method, the respired carbon dioxide being absorbed in barium hydroxide. All determinations were carried out at room temperature, which remained nearly constant at 22° C. Determinations were made at 24-hour intervals for a period of seven days and were in duplicate.

To count the lenticels, the tubers were boiled and the entire skin removed, cut into pieces approximately 2 cm. square, placed on a moistened glass plate and held up to the light. The lenticels were then easily counted, after which the pieces of skin were laid on cross-section paper and their area estimated.

The data obtained are presented in table I. In general, the amount of carbon dioxide respired per kilogram of tubers increases from large to small tubers. The medium sized Green Mountain tubers are an exception, probably owing to the difficulty of selecting a truly median class of tubers. The relationship between surface area and size shows a similar trend. These results agree well with those of HOFFMAN (1), who came to the conclusion that the higher respiratory rate of the small tubers was due to their greater surface

area in proportion to volume. It will also be noted that the number of lenticels per kilogram increases from large to small tubers. There thus appears to be a relationship between the rate of respiration, surface area, and number of lenticels per kilogram of tubers.

TABLE I

MILLIGRAMS CARBON DIOXIDE RESPIRED AT 22° C.; SURFACE AREA AND NUMBER OF LENTICELS FOR LARGE, MEDIUM, AND SMALL POTATO TUBERS

Size	Number of tubers	Carbon dioxide (mg.)			Surface area in sq. cm. per kilo	Lenticels	
		Per kilo per hour	Per sq. cm. per hour	Per lenticel per hour		Per kilo	Per sq. cm.
		Early Ohio					
Large	4	34.35	0.0296	0.0126	1162	2731	2.35
Medium	4	42.66	0.0312	0.0123	1368	3475	2.54
Small	6	58.02	0.0329	0.0075	1765	7713	4.37
Average		45.01	0.0314	0.0097	1432	4640	3.09
		Irish Cobbler					
Large	4	15.75	0.0128	0.0061	1233	2564	2.08
Medium	4	20.69	0.0149	0.0053	1391	3895	2.80
Small	6	21.03	0.0124	0.0039	1702	5429	3.19
Average		19.16	0.0135	0.0049	1442	3963	2.69
		Green Mountain					
Large	4	15.58	0.0118	0.0041	1321	3778	2.86
Medium	6	14.99	0.0116	0.0033	1296	4601	3.55
Small	6	17.52	0.0104	0.0032	1687	5466	3.24
Average		16.03	0.0112	0.0035	1435	4615	3.22

Carrying the analysis somewhat further, it is seen from table I that the amount of carbon dioxide respired per square centimeter of surface shows no consistent differences between large and small tubers, despite the much larger volume of tissue dependent upon a unit of surface for gas exchange with the surrounding air in the case of the large tubers. The amount of carbon dioxide respired per lenticel, however, decreases from large to small tubers. This is no doubt due to the higher number of lenticels per unit of surface in the

small tubers, as is shown in the last column of the table. It is also probable that the lenticels of the large tubers have a somewhat greater diameter than those of the small tubers.

These observations indicate that the lenticels are the chief avenue of gas exchange between the internal tissues of the potato tuber and the surrounding air. The work of SMITH has shown that the internal tissues offer considerable resistance to the rapid movement of gases into or out of the tuber, which probably largely accounts for the low rate of gas exchange in the large tubers. It hardly seems probable that the smaller number of lenticels per unit of area on the large tubers could be the factor slowing down the respiration rate in the large tubers, since LUTMAN (**2**) has shown that the lenticels are capable of accommodating a higher rate of gas exchange than normally occurs. He cut tubers in half, hollowed out the halves, tied them together and sealed the union with vaseline. Owing to wound response these tubers respired at a much higher rate than the normal checks; yet the gas exchange was all through the periderm which had not been injured except at the point of the union sealed by vaseline.

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RELATION OF PINOXYLON DAKOTENSE KNOWLTON TO PROTOPICEOXYLON GOTHAN

RICHARD KRÄUSEL

A redescription of *Pinoxylon dakotense* Knowlton, a coniferous wood of the Lakota sandstone (Lower Cretaceous, Black Hills), has been given by READ (**6**). He finds that it is no *Pinus* but belongs to *Protopiceoxylon* Gothan. Thus he confirms the supposition already made by me in 1919. It is surprising that READ does not make mention of my paper (**1**), as it affords a critical list of all the fossil coniferous woods (with exception of *Araucarioxylon* Kr.) known up to that time. As to *Pinoxylon dakotense*, I wrote, "Dieses angeblich zu *Pinus* gehörende Holz steht in enger Beziehung zu *Protopiceoxylon*."

In that same paper, one might have found that the description of *Protopiceoxylon salisburioides* (Göpp.) Kr. is by no means "brief and without illustrations" (READ). READ refers only to my preliminary statement of 1913 (**2**), overlooking the full and richly illustrated account given in 1920 (**3**). There it is shown that the peculiar anatomical features of that wood from the Miocene lignite (browncoal) (for example, resin ducts, strongly pitted walls of the rays, etc.) are traumatic, and that the wood is of true abietinean affinity. Therefore it was excluded from Mesozoic *Protopiceoxylon* species and the name *Cedroxylon salisburioides* was proposed.

May I mention that twice (**1, 4**) I considered the bearing of wood anatomy and of fossil woods on the phylogeny of conifers? I came to the conclusion that JEFFREY was wrong and that the Araucarieae are older than the Abietineae. But we both were wrong. Abietineae and Araucarieae are groups of living (and extinct) plants, characterized by distinct anatomical and morphological features. If we find a Mesozoic or Paleozoic conifer with araucarian wood but abietinean cones, or vice versa (and I think they will be found one day), it would be wrong to include them in the Abietineae (or Araucarieae). They form, on the contrary, a new, extinct group of conifers, from which

both the living Araucarieae and Abietineae might have descended. Since only the anatomical side of the problem is known, we are not justified in speaking (as JEFFREY and I and other writers have) of the age of Abietineae and Araucarieae, but only of the age of the one or other type of wood. And here we may state that among fossil conifers the araucarian type of wood is the older one. It should not be forgotten, however, that the Mesoxyleae of the Paleozoic are in no way connected with true conifers. But even among them we find *Abietopitys*, a form with "abietinean" pitting of the rays (**5**).

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BRIEFER ARTICLE

ROOT FORMATION FROM LEAF CUTTINGS

While experimenting with various kinds of leaves and portions of leaves which might be used as cuttings at different times during the year,

FIGS. 1–3.—Fig. 1, leaf cutting put in soil October 2; diagram made November 24; fig. 2, segment cutting put in soil October 2; diagram made November 24; fig. 3, leaf cutting showing tuber-like roots.

well-rooted cuttings have been secured by using single leaves of *Dahlia variabilis* and imbedding 20 mm. of the petioles in sand (fig. 1). Roots have also been developed at the same time of year, using only the termi-

nal segment (fig. 2). The cuttings rooted in eight weeks, and about two weeks later the roots were beginning to thicken (fig. 3). The plants developed and blossomed the next summer. Since it is necessary to take only a few leaves from the plant late in the fall to start an equal number of plants, this method does not greatly interfere with the storage of food material in the root.

It is hoped to try this experiment at different times during the year, and at the same time to analyze the food content, together with the internal structure of both the petiole and the base of the terminal segment of the leaf.—EDITH A. ROBERTS AND JULIA R. LAWRENCE, *Department of Botany, Vassar College, Poughkeepsie, New York.*

CURRENT LITERATURE

Announcement

Under the new arrangement for quarterly publication the Botanical Gazette can use two or three longer papers in each issue, such papers being accepted at the discretion of the editors. This plan removes the ruling regarding page limits for manuscripts.

Papers being accepted as this note goes to press will appear in the coming June issue.

BOOK REVIEWS

Chemistry and technology of oils and fats

The second edition of UBBELOHDE'S *Handbuch der Chemie und Technologie der Öle und Fette* appears in four volumes (two of which are in two volumes each, making six volumes), under the editorship of Dr. H. HELLER of Magdeburg. The first volume (pp. xvi+791) discusses the chemistry and technology of the oils, fats, and waxes. The second volume (pp. xii+824) is divided into two parts, the first of which deals with the chemistry and technology of vegetable oils and fats. The second part is still unpublished. Volume III, also in two parts, treats in part 1 (pp. xx+392) the chemistry, analysis, and technology of the fatty acids, glycerol, and Turkey-red oils; and in part 2 (pp. xx+752) the chemistry and technology of soaps and washing materials. The fourth volume (pp. xiv+798) is devoted to the oxidized, polymerized, and reduced fatty acids, and the waxes.

This review will consider only the first part of volume II,[1] on the vegetable oils and fats, which has just been published. The work falls into four sections, as follows: strong drying oils; weak drying oils; non-drying oils; and the solid vegetable fats. In treating these materials, the arrangement is based on the plant families, passing in order from the lowest to the highest families in which the given type of oil is found. The section on strong drying oils begins with those found in the Taxaceae and Pinaceae, and ends with those of the Compositae, such as burdock seed oil, thistle oil, sunflower oil, dandelion oil, cocklebur oil, etc. Similarly the section on weak drying oils begins with those of the Gramineae (corn oil), proceeds to the oils of such families as the Ranunculaceae, Cruciferae, Leguminosae, Euphorbiaceae, Malvaceae, Umbelliferae, etc., and

[1] HELLER, HANS. UBBELOHDE'S Handbuch der Chemie und Technologie der Öle und Fette. Vol. II, part 1. Chemie und Technologie der pflanzlichen Öle und Fette. 8vo. pp. xii+824. Hirzel. Leipzig. 1932.

ends with weak drying oils of such composites as artichoke, lettuce, and goldenrod. The sections on non-drying oils and solid fats begin with those of the fungi.

The method of arrangement gives the work an encyclopedic character; it is an excellent dictionary of the vegetable oils and fats. The table of contents lists the page references in four columns, headed by the names of the four types of vegetable oils and fats, and the first column lists the names of the plant subkingdoms and families from Thallophyta to the highest family in the ENGLER and PRANTL system. The Bryophyta alone have no representatives in the list.

There is a brief appendix giving the results of a few papers published from 1929 to 1931 that were not seen in time to include in the main text. The work of almost 1500 investigators has been drawn upon for the data included in this part 1 of volume II. It is on the whole a very valuable summary, and will be indispensable to those who wish information on the distribution of the vegetable oils and fats in nature. It will also be a source of information as to the characteristics of the oils. In tabulated form it presents such information as is obtainable as to the specific gravity, melting points, solidification points, refractive indices, acid numbers, saponification numbers, iodine numbers, Reichert-Meissl numbers, acetyl values, and percentage of unsaponifiable fats, for the oils and fats listed. One can therefore obtain in very concise form whatever is known concerning almost any vegetable oil or fat that is mentioned in the literature. —C. A. SHULL.

A taxonomic study in Potamogeton

In the past, misdeterminations of American species of *Potamogeton* have been almost as common as the mispronunciation of the generic name. A recent notable work by FERNALD[2] should do much to clarify our knowledge of these species and to render a proper identification reasonably certain. For some fifteen years FERNALD has studied, although rather intermittently, the genus in a monographic way, his investigations including the first-hand examination of "approximately 12,000 sheets." It is cause for rejoicing among all who have the welfare of taxonomic conservatism at heart when an authority of such exacting scholarship devotes his best thought and effort to the revision of such a group. Especially is this true of *Potamogeton*, "for, unfortunately," as we find stated in quite another connection, "the group has been so generally avoided by botanists of accurate minds and scholarly temperaments, that it has in recent years drifted largely into other hands and has been treated in an impressionistic and slipshod manner almost unbelievable."

[2] FERNALD, M. L., The linear-leaved North American species of *Potamogeton*, section Axillares. 4to. pp. 183. Pls. 40, maps 31. Memoirs American Academy of Arts and Sciences. Vol. XVII. part I. 1932; reprinted in Memoirs of the Gray Herbarium of Harvard University, no. III. 1932.

The text falls into five major divisions, entitled: introduction; diagnostic characters (fruits, spikes, sepaloid connectives, peduncles, stipules, leaves, nodal glands, winter buds or hibernacula, stems, and rhizomes); geographic distribution of the subsections and species (broad distribution of the subsections, interrupted ranges in North America); synoptic treatment of the North American species (material studied, illustrations, maps, the classification of North American pondweeds, key to the indigenous linear-leaved North American species of *Potamogeton* § Axillares, synopsis of species); and list of exsiccatae cited. The introduction gives an extended historical survey with many pointed criticisms and observations. The main body of the text abounds in distributional, geological, and other types of data and the individual taxonomic treatments are supplemented with numerous extended discussions that greatly enhance their value. The geographic range of various species and varieties is shown graphically by dotted maps. Forty full-page plates of half-tone illustrations serve to give additional guidance in the interpretation of the text. Generally speaking, these represent a high standard of excellence. The "list of exsiccatae cited" is extensive but does not include, as one might infer from the title, the numerous unnumbered specimens cited in the text. Occasional slips occur, but fortunately these are not abundant.

FERNALD'S memoir is characterized throughout by an attempt to cut loose from "the impressionistic," the subjective, one might even say "the psychological" bases for delimiting species and subspecific entities. It represents a commendable effort to associate taxonomic units with tangible morphological characters; in short, to produce a treatment resting upon a firm objective foundation.—E. E. SHERFF.

Manual of bryology

A volume has just been published under the editorship of FR. VERDOORN,[3] in collaboration with fifteen other contributors. It represents an effort to bring together in one volume the most recent research on the Bryophyta in several fields of investigation. The first two chapters deal with the morphology and anatomy of the mosses and liverworts respectively, the former being treated by R. VAN DER WIJK and the latter by H. BUCH. The treatment in these chapters is somewhat general but adequate for a volume of this type. It is to be regretted that these sections are not more completely illustrated. The present status of experimental morphology is treated by BUCH in chapter III. The fourth chapter deals with germination of spores and development of protonema, presented by G. CHALAUD.

The ecological aspects of the Bryophyta are treated in several separate chapters, which, curiously enough, are not arranged in sequence. Chapter V, on the

[3] VERDOORN, FR. (editor) et al., Manual of bryology. pp. viii+485. 129 illus. Martinus Nijhoff. The Hague. 1932.

association of bryophytes with other organisms, is presented by G. NICHOLAS; chapter X, covering geographical distribution, is written by TH. HERZOG; and quaternary distribution is treated by H. GAMS. Other ecological phases dealing with moss societies are presented in chapter XII by the same author, with a concluding chapter on ecology by P. W. RICHARDS.

The cytological and genetical aspects of the bryophytes are very ably handled in chapter VI on cytology, by J. MOTTE; chapter VII, dealing with nuclear phases, by K. HOEFER; and chapter IX on genetics, by F. VON WETTSTEIN. The section devoted to physiology was prepared by A. J. M. GARJEANNE. The concluding chapters deal with the taxonomy and phylogeny of the bryophytes. The classification of the mosses is presented by A. N. DIXON, that of the hepatics by FR. VERDOORN, while the phylogenetic aspects are presented by W. ZIMMERMANN.

While it is obviously impossible to treat such a wide array of subjects adequately within the scope of a single volume, it is nevertheless encouraging that a manual of this type has been attempted. It will serve as a valuable handbook for all those interested in bryology; and since there are adequate bibliographies or reviews of the significant literature under each section, it will be very helpful to workers in this field in making contacts with original sources.—H. E. HAYWARD.

College textbook of botany

MOTTIER has revised and amplified his *College textbook of botany for first-year students*, so that it now appears as a *Textbook of botany for college students*.[4]

Structure, function, and life history are described in concrete form, while touches of human interest brighten the presentation. The work begins with the life history of a seed-bearing plant, following which there is a chapter on the cell. The author's long series of researches on cell structures, dating from the nineties, when he was studying with STRASBURGER, have enabled him to write a much more authoritative account than could be expected from textbook writers whose first-hand knowledge of cell structures is limited or even lacking altogether. In succeeding chapters, leaf, stem, and root are described in detail, the structure and function being correlated throughout. In addition, there is a special chapter on the movement of water and food in plants. Seeds and seedlings, utilization of food, energy release, and growth and response to stimuli are the subjects of several succeeding chapters.

The foregoing features occupy 185 pages; the following 342 pages are devoted to plants arranged in taxonomic sequence from the myxomycetes to the angiosperms. The book closes with chapters on the dispersal of seeds and fruits, geographical distribution of plants, and heredity.

[4] MOTTIER, D. M., A textbook of botany for college students. 8vo. pp. x+601. Figs. 542. P. Blakiston's Son and Co. Philadelphia. 1932.

From the standpoint of the reviewer, the treatment of alternation of generations is inadequate, for he believes that wherever there is sexuality there must be an alternation of generations. In an elementary book this subject might properly begin with the bryophytes; but, in an advanced college text, the origin and evolution of alternation should be considered in the thallophytes since groups above this level illustrate only the evolution of an already well established sporophyte and the reduction of the gametophyte. We all use the term sporophyte and gametophyte; but the fact that they are misnomers in many of the thallophytes indicates that they do not correspond to fundamentals in the phenomenon of alternation.

The presentation throughout is what would be expected from a teacher who has long been an active investigator. There are 542 illustrations. The author draws so well and so accurately that the extensive borrowing of illustrations, a few of which are incorrectly credited, seems unfortunate. The originals, especially in pteridophytes and spermatophytes, are excellent.

At the end of each chapter there are stimulating topics for review and discussion. The whole presentation is so practical and has such an atmosphere of the laboratory about it, that no specific laboratory directions are necessary —C. J. CHAMBERLAIN.

Handbook of plant analysis

The first of KLEIN'S volumes on plant analysis was noted in this journal[5] some months ago. The second volume has now appeared,[6] and it is evident that the work as a whole is to be a most useful compilation of methods. This volume takes up the analysis of plant tissues for inorganic and organic constituents. The first section outlines the methods for determination of the inorganic substances in four groups: (1) the most important cations and anions, and occasionally occurring elements; (2) inorganic nitrogenous ions; (3) preparation and analysis of plant ash; and (4) gas and micro-gas analysis.

The section on organic substances covers the most important of the organic plant constituents. The subsection headings are as follows: alcohols; aldehydes and ketones; phenols and quinones; organic acids; phosphoric acid esters of carbohydrate metabolism; general consideration of "lipoids"; fats and waxes; phosphatides; phytosterols; sugar alcohols (polyhydric); simple carbohydrates (mono- and di-saccharides); characteristics, preparation, and determination of individual sugars; and polysaccharides (inulin, glycogen, starch).

At the close of each chapter a list of plant families is given, in which each of the substances treated occurs. This statement of the distribution of compounds should be helpful. While it is very difficult to make such lists even tolerably complete, one can obtain a fair idea of the distribution of certain constituents by

[5] BOT. GAZ. **92**:332–333. 1931.

[6] KLEIN, G. Handbuch der Pflanzenanalyse. Vol. II. 8vo. pp. xii+973. Julius Springer. Vienna. 1932.

consulting these condensed statements. Occasionally very erroneous statements are made regarding the distribution of substances in nature. For instance, the statement has recently been made[7] that inulin had been found thus far only in two plant families. HAAS and HILL, however, mention a dozen or more families, and KLEIN'S *Handbuch* mentions more than twenty families in which inulin has been found, not counting the ferns, fungi, and algae.

Among the contributors who have made this excellent work possible are men who have contributed largely to our knowledge of the substances they treat. RIPPEL, PRIANISCHNIKOW, NEUBERG, WEHMER, WINTERSTEIN, and PRINGSHEIM are among those who have worked with KLEIN. One has confidence in a work which is produced by the cooperative efforts of expert plant chemists. The completed work is to consist of four volumes. The remaining ones will be awaited with much interest.—C. A. SHULL.

Microchemistry

A translation of EMICH'S manual of microchemistry has been made by SCHNEIDER.[8] The book in the German passed through two editions. The English edition is brought up to date by the inclusion of material published since the second German edition went to press. Also, there are included references to American literature and sources of supply of apparatus.

The book is divided into two main parts. The first considers apparatus and methods, and the second describes practice exercises. Each of these parts is further divided into a qualitative section and a quantitative section. Some of the subjects discussed in the first part are: the microscope, including the polarizing microscope; reagents; treatment of precipitates; recrystallization; boiling point determination and fractionation; sublimation; schlieren observations; qualitative microanalysis with small electrodes; and mounting permanent microchemical preparations. The second part of the book, including the section of spot analysis, describes 84 exercises, applying the methods described in a general way in the first part. These exercises have mainly to do with the detection and the quantitative determination of the amount of various ions and compounds. There is an inorganic section and an organic section. There are also exercises on the use of the microscope, including the polarizing microscope.

The book is mainly of value to the chemist, and he should find it of very great help. The author has not tried to include botanical microchemistry, such as given in the standard textbooks of TUNMANN and MOLISCH. Yet botanists, especially plant physiologists, will find certain sections of great value, especially

[7] Amer. Jour. Bot. **19**:187. 1932.

[8] EMICH, FRIEDRICH, Microchemical laboratory manual, with a section on spot analysis by FRITZ FEIGL. Trans. by F. SCHNEIDER. pp. xvi+180. Figs. 88. John Wiley and Sons. New York. 1932.

those having to do with the microscope and the polarizing microscope, reagents, sublimation, and some of the qualitative exercises on the inorganic cations and anions. But there is still great need of a textbook in English on botanical microchemistry.—S. V. EATON.

Tropical woods

A publication[9] which is issued four times a year by the Yale School of Forestry, for the subscription price of $1.00, contains very interesting articles dealing with anatomy, morphology, taxonomy, and ecology of tropical woods, giving proper attention to their usefulness. It includes not only original articles but also book reviews on important literature pertaining to tropical silviculture. Up to now twenty-nine numbers have appeared, of which the last one is for March 1, 1932. The wealth of information which this magazine contains may be judged from a few titles taken from recent issues.

KANEHIRA, RYOZO, Forest trees of Micronesia.
DESCH, H. E., Significance of numerical values for cell dimensions.
CHATTAWAY, M. N., Proposed standards for numerical values used in describing woods.
RECORD, SAMUEL J., Brazilian kingwood (*Dalbergia cearensis*).
STANDLEY, PAUL C., Mexican and Central American species of *Pterocarpus*.
———, Two new trees from South America.
RECORD, SAMUEL J., Notes on Brazilian timbers.
PITTIER, H., Note on *Escallonia tortuosa* H.B.K.
WILLIAMS, L., Forests of northeastern Peru.
MOLDENKE, HAROLD N., Three new species of *Aegiphila* from Central America.
DAHLGREN, B. E., Tupi manner of forming names of trees.
MACBRIDE, J. FRANCIS, South American Viburnums incorrectly described as new species of *Cornus*.
AITKEN, J. B., Wallabas of British Guiana.
COOPER, G. PROCTOR, Expavé (*Anacardium rhinocarpus* DC).
REYES, LUIS J., Apitong (*Dipterocarpus* spp.) of Northern Negros.
HÉDIN, L., Commercial mahoganies of French Cameroon.
RECORD, SAMUEL J., Notes on Australian woods.—A. C. NOÉ.

Waste products of agriculture

A volume of considerable interest to the practical botanist and agriculturist has been written by HOWARD and WAD.[10] The book is concerned with the problem of the utilization of the by-products of the farm in increasing the fertility of the land. The authors are well equipped to discuss such a subject, for they are

[9] Tropical woods, edited by SAMUEL J. RECORD, School of Forestry, Yale University.

[10] HOWARD, ALBERT, and WAD, Y. D., The waste products of agriculture and their utilization as humus. pp. ix+167. Oxford University Press. 1931.

thoroughly familiar with the intensive systems of agriculture of the Orient. It is only there that much has been done in making use of the wastes of the farm. The senior author has been studying the problems of crop production in India for 26 years.

The main topics discussed are: a comparison of the agricultural systems of the Occident and the Orient; the sources of organic matter and its relation to soil fertility; the Indore process for the manufacture of humus, with the main factors involved and the practicability of using the process in other areas.

The book should do much to stimulate interest in the more complete and efficient utilization of the by-products of the farm in the United States and other countries where the extensive system of agriculture is used. One of the main difficulties in the way of adopting the Indore process in these countries is, of course, the scarcity of labor. But, as pointed out by the authors, mechanization should solve this problem. Another difficulty would probably be the opposition of the manufacturers of artificial fertilizers. But if one or two agricultural experiment stations would demonstrate the value of the process in increasing crop yields, there might be a gradual adoption of the plan in spite of this opposition. This would not mean that artificial fertilizers would not be used, for the best results are obtained when the humus and artificial materials are combined.—S. V. EATON.

Megaspores from coal seams

In recent years very important studies have been made of the spores found in some German and Polish coal beds.[11] The method which has been successfully introduced for the preparation of pollen diagrams in peat bogs has now been used for coal seams, and a great many different types of megaspores have been recorded for various seams and at various horizons in the seams. It is very difficult to determine to what species of fossil lycopods or Equisetales these spores belong, and therefore it is necessary to establish form types of these spores either

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[11] ZERNDT, J., Megasporen aus einem Flöz in Libiaz (Stéphanien). Extrait du Bulletin de l'Académie Polonaise des Sciences et des Lettres. Classe des Sciences Mathématiques et Naturelles. Série B: Sciences Naturelles (1). 8vo. pp. 39–70. Pls. 1–8. Cracow. 1930.

———, Triletes giganteus, n. sp., eine riesige Megaspore aus dem Karbon. Extrait du Bulletin de l'Académie Polonaise des Sciences et des Lettres. *Ibid.* pp. 71–79. Pls. 9–11. Cracow. 1930.

———, Megasporen als Leitfossilien des produktiven Karbons. Extrait du Bulletin de l'Académie Polonaise des Sciences et des Lettres. Classe des Sciences Mathématiques et Naturelles. Série A: Sciences Mathématiques. 8vo. pp. 165–183. Pls. 3–10. Cracow. 1931.

POTONIÉ, R., Sporenformen aus den Flözen Ägir und Bismarck des Ruhrgebietes. Neues Jahrbuch für Mineralogie etc. Beil. **67**:Abt. B. 8 vo. pp. 438–454. Pls. 14–20. Berlin. 1932.

under the genus names Triletes or Sporonites. It is hoped that gradually it may be possible to identify the relation of spores to known plant species. Meanwhile a large and desirable amount of information has been made available about the spores themselves.—A. C. Noé.

Physical chemistry of sexuality

Joyet-Lavergne has written a significant book[12] on the physical chemistry of sexuality, the volume being one of the Protoplasma monographs. It is a general treatise on the subject, including both plant and animal work. The nature of the material of the book is indicated by reference to the titles of some of its 14 chapters. There are chapters on various sexual differences, morphological, physiological, and physico-chemical. Other chapters treat the genital hormones, the chemical differences characteristic of sex, cytoplasmic sexuality, intersexuality, the change of sex, the physico-chemical conception of sexuality, various theories on the problem of sexuality, intersexuality, and metabolism in relation to sexuality. In the latter chapter the work of Riddle on the metabolism of the pigeon is discussed. The book gives a critical, unbiased discussion of the whole field of sexuality. There is a bibliography of about 50 pages, containing hundreds of references to American and English work. The volume will be welcomed by the botanist and zoologist, especially those interested in genetics. —S. V. Eaton.

Vegetation of the Canary Islands

The position of the Canary Islands, so near the coast of Africa and yet so different from it in vegetation, has for many decades made their flora of special interest to botanists. By their Tertiary relicts and their numerous endemics these islands seem almost to belong to another world. Interest shown concerning the vegetation in past years will be greatly augmented by an elaborate treatise on the ecology and distribution of the elements of the flora which has recently appeared.[13] The author has discussed in detail the age, the geology, and the climate of the group, together with the influence of these factors on the flora as a whole. Each of the seven islands is then considered individually with respect to the peculiarities of its flora, and finally the individual species are discussed and illustrated. The large size of the volume (9×12 inches) permits the reproduction of the numerous photographs in elaborate and unusually fine plates. The work has been so carefully done, the descriptions are so thoroughly organized, and the illustrations are reproduced in such excellent plates that a graphic picture of a unique flora has resulted.—G. D. Fuller.

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[12] Joyet-Lavergne, Ph., La physico-chimie de la sexualité. 8vo. pp. xi+457. Gebrüder Borntraeger. Berlin. 1931.

[13] Burchard, Oscar, Beiträge zur Ökologie und Biologie der Kanarenpflanzen. Bibliotheca Botanica **98**:1–262. Pls. 78; map. Erwin Nägele. Stuttgart. 1929.

Principles of soil microbiology

The excellent text by WAKSMAN[14] dealing with the principles of soil microbiology has been extensively rearranged and revised. The new discussion on mycorrhizal fungi is valuable. Of especial interest to plant physiologists are the new chapters on the rôle of microorganisms in the decomposition of plant residues and the formation of peat. The discussion of the chemical dynamics of the soil is well done. This book is of considerable size (894 pages), and is an exhaustive treatment of the subject.—R. B. HARVEY.

[14] WAKSMAN, S. A., Principles of soil microbiology. 2d ed. 8vo. pp. xxviii+894. Williams and Wilkins Co. 1932.